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A Summary of Current Program and  
Preliminary Report of Progress

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DAIRY RESEARCH

of the

United States Department of Agriculture  
and Cooperating Agencies

This progress report of U.S.D.A. and cooperative research is primarily a tool for use of scientists and administrators in program coordination, development, and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on U.S.D.A. and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having an interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of U.S.D.A. and cooperative research issued during the last year. Current agricultural research findings are also published in the monthly U.S.D.A. publications, Agricultural Research, Agricultural Marketing, and The Farm Index.

UNITED STATES DEPARTMENT OF AGRICULTURE

Washington, D. C.

December 1, 1963





## ADVISORY COMMITTEES

The research program of the Department of Agriculture is reviewed annually by the following advisory committees:

1. Farm Resources Research
2. Utilization Research and Development
3. Human Nutrition and Consumer Use Research
4. Marketing Research and Service
5. Agricultural Economics Research
6. Forestry Research
7. Animal and Animal Products Research
8. Cotton and Tobacco Research
9. Grain and Forage Crops Research
10. Horticultural Crops Research
11. Oilseed, Peanut and Sugar Crops Research

## ORGANIZATIONAL UNIT PROGRESS REPORTS

The source materials used by the advisory committees are of two types. First, there are Organizational Unit Reports that cover the work of the Divisions or Services listed below. The number prefixes refer to advisory committees listed above that review all of the work of the respective Divisions or Services.

### Agricultural Research Service (ARS)

- 1 - Soil and Water Conservation
- 2 - Utilization -- Eastern
- 2 - Utilization -- Northern
- 2 - Utilization -- Southern
- 2 - Utilization -- Western
- 3 - Human Nutrition
- 3 - Clothing and Housing
- 3 - Consumer and Food Economics
- 7 - Animal Husbandry
- 7 - Animal Disease and Parasite

### Agricultural Marketing Service (AMS)

- 4 - Market Quality
- 4 - Transportation and Facilities

### Economic Research Service (ERS)

- 4,5 - Marketing Economics
- 5 - Farm Production Economics
- 5 - Resource Development Economics
- 5 - Economic & Statistical Analysis
- 5 - Foreign Development and Trade Analysis
- 5 - Foreign Analysis Division

### Other Services

- 1 - Soil Conservation Service (SCS)
- 4,5 - Farmer Cooperative Service (FCS)
- 4,5 - Statistical Reporting Service (SRS)
- 6 - Forest Service (FS)

Three organizational unit reports are not reviewed in entirety by any one committee. All of the information in them is included in the subject matter reports.

Agricultural Research Service (ARS)

Agricultural Engineering  
Crops  
Entomology

SUBJECT MATTER PROGRESS REPORTS

The second type of report brings together the U.S.D.A. program and progress for the following commodities and subjects:

- |  |                                    |
|--|------------------------------------|
| 1 - Cross Commodity Research of          | 8 - Cotton and Cottonseed          |
| Agricultural Engineering, Crops,         | 8 - Tobacco                        |
| & Entomology Research Divisions          | 9 - Grain and Forage Crops         |
| 3 - Rural Dwellings                      | 10 - Citrus & Subtropical Fruit    |
| 6 - Forestry (Other than Forest Service) | 10 - Deciduous Fruit & Tree Nut    |
| 7 - Beef Cattle                          | 10 - Potato                        |
| 7 - Dairy                                | 10 - Vegetable                     |
| 7 - Poultry                              | 10 - Florist, Nursery & Shade Tree |
| 7 - Sheep and Wool                       | 11 - Oilseed and Peanut            |
| 7 - Swine                                | 11 - Sugar                         |
| 7 - Cross Species & Miscellaneous        |                                    |
| Animal Research                          |                                    |

A copy of any of the reports may be requested from Max Hinds, Executive Secretary, Animal and Animal Products Research Advisory Committee, Agricultural Research Service, U. S. Department of Agriculture, Washington 25, D. C.

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## INTRODUCTION

This report on dairy research covers work directly related to the production, processing, distribution, and consumption of milk and its products. The information has been assembled from the organizational unit reports of the several divisions. This report does not include extensive cross-commodity work, much of which is basic in character, which contributes to the solution of not only dairy problems but also to the problems of other commodities. Progress on cross-commodity work is found in the reports of the several divisions such as Soil and Water Conservation, Human Nutrition, Transportation and Facilities, Farm Production Economics, Foreign Development and Trade Analysis, and Cross-Species and Miscellaneous Animal Research.

This report is devoted to the 16 "problem areas" shown in the table of contents. For each area there is a statement of (1) the Problem, (2) the USDA Program, (3) A summary of Progress during the past year on USDA and cooperative work, and (4) A list of Publications resulting from USDA and cooperative work.

Dairy research can be divided into three major categories, i.e., that supported by (1) Federal funds appropriated to the research agencies of the United States Department of Agriculture, (2) Federal and State funds appropriated to the 53 State Agricultural Experiment Stations, and (3) private funds allotted, largely by the dairy industry, to research carried on in private laboratories or to support of State Station or USDA work. For all three categories it is estimated that about 2,100 scientists are engaged in research dealing specifically with the production, processing, distribution, and consumption of dairy animals, milk, and its products. Support of their work involves an annual expenditure of between 50 and 55 million dollars. This amounts to about 0.8 percent of the cash farm receipts from the sale of dairy animals for slaughter and dairy products and about 0.4 percent of the retail value of the same items. Of the 2,100 scientists engaged in dairy research, approximately 14% are employed by the Department of Agriculture, 21% by the State Experiment Stations, and 65% by other universities, foundations, and private industry.

### Research by USDA

Farm research pertaining to dairy is conducted in the Agricultural Research Service divisions of Agricultural Engineering, Animal Disease and Parasite, Animal Husbandry, and Entomology. The work comprises investigations of breeding, physiology, nutrition, diseases and parasites, housing and management of dairy animals, and sanitary handling and storing of milk until it leaves the farm. The work involves 190 professional man-years of scientific effort which includes research on cattle diseases and parasites that is applicable to beef cattle, also.

Nutrition, consumer, and industrial use research pertaining to dairy is conducted in the Agricultural Research Service divisions of Human Nutrition, Consumer and Food Economics, and Eastern Utilization. The work comprises investigations of composition and nutritive value; physiological availability of nutrients and their effects; new and improved methods of preparation, preservation, and care in homes, eating establishments and institutions; and with the processing phase involving pasteurization, bottling, separation of cream and skim milk; manufacture into products such as butter, powder, cheese, concentrated forms, ice cream, and numerous specialty items. Also, it is concerned with improved equipment and processes. The work in these divisions involves 100 professional man-years of scientific effort.

Marketing and economic research pertaining to dairy is carried on within four Services: Agricultural Marketing Service, Economic Research Service, Farmer Cooperative Service, and Statistical Reporting Service. The work comprises (1) physical and biological aspects of assembly, packaging, transporting, storing and distribution; (2) economic aspects of marketing costs, margins and efficiency, market potential, supply and demand, and situation and outlook; (3) cooperative marketing, and (4) consumer acceptance studies. The divisions in which the work is conducted are: Market Quality, AMS; Transportation and Facilities, AMS; Marketing Economics, ERS; Economic and Statistical Analysis, ERS; Marketing Division, FCS; Standards and Research, SRS. The scientific effort involved by these divisions amounts to 11 professional man-years.

#### Interrelationships among Department, State and Private Research

A large part of the Department's research is cooperative with State Experiment Stations. Many Department employees are located at State Stations and use laboratory and office space close to or furnished by the Station. Cooperative work is jointly planned, frequently with the participation of representatives of the producers or industry affected. The nature of cooperation varies with each study. It is developed so as to fully utilize the personnel and other resources of the cooperators which frequently includes resources contributed by the interested producers or industry.

Including both cooperative and State Station projects dairy research is carried on by 52 State Experiment Stations. The types of work to which the largest amount of effort is devoted include nutrition and management, diseases, breeding, physiology, utilization and marketing. There is regular exchange of information between Station and Department scientists to assure that the programs complement each other and to eliminate unnecessary duplication.



Privately supported dairy research emphasizes the solution of scientific production, processing, and marketing problems. Much of it utilizes the results of basic work done by State Station and Department scientists.

A number of companies make application of basic research developed through public research on products intended for prevention, control, or treatment of diseases, parasites and insects; however, final evaluation is often done cooperatively with public agencies. Most of the identification and classification of insects, diseases and parasites is done by public institutions but the information is available for use by individuals and firms.

About 1/3 of the industry research effort in the dairy industry is in the utilization field. Very little of the work is basic and where it is, the results are usually patented. In applied research the major activities are in cost saving, container testing and low calorie product formulation. Public research was used to develop the procedure for removal of strontium-90 from milk.

The contributions of dairy producers and industry to the work of the State Stations and the Department have been an important factor in the success of their research programs. Producers offer herds and facilities for testing products and practices used in production. Likewise, processors and retailers offer facilities and products for use by public research agencies. Many problems in the economics of marketing cannot be transferred to a laboratory, experimental plot, or other simulated situation. The results of economic research conducted cooperatively is of great value to industry, especially in cases where public research can provide comparison and analysis. Even large firms that have a research staff do not have access to the plants and records of competitors.

#### Examples of Recent Research Accomplishments by USDA and Cooperating Scientists

Ventilation of livestock buildings. Research in cooperation with State Experiment Stations has obtained much needed basic data on the heat and moisture given off by cattle, hogs, and poultry, and on the influence of building environment on production and feed consumption. The heat and moisture dissipation data are considered basic design data for ventilation systems of poultry, dairy, and swine buildings. They appear in design handbooks including the 1962 Guide and Data Book of the American Society of Heating, Refrigeration, Ventilating, and Air Conditioning Engineers, and are used by makers of ventilating equipment, prefabricated buildings and package buildings as well as by specialists advising farmers on their own construction. Building improvements resulting from the above research have contributed to the substantial rise in efficiency of livestock production that has occurred during the past decade.

New DHIA sire evaluation. A new method is being used by the USDA for DHIA sire evaluations. The dam-daughter comparison proved-sire record has been replaced by sire summaries using daughter-herdmate comparisons. The new method eliminates much of the bias and error inherent with the older technique and provides dairymen with a greatly improved sire record, particularly for sires used in artificial insemination. New data processing methods will permit computation and issuance of quarterly instead of once a year sire summaries. In addition, cows with superior breeding values will be identified for use in making matings to produce future bulls.

Microbes help with pesticide residue problems. Ruminal protozoa, cultured as individual species, offer definite promise as a screening technique for determining if pesticides will leave residues in meat and milk of cattle and sheep. Ruminant animals possess large numbers of microorganisms in their digestive system, particularly the rumen which appears to be the natural site for the microbial degradation of complex compounds such as pesticides. Pesticides, if degraded in this manner, will not produce residues in the meat even though they are consumed with the feed. Ruminal protozoa were found to metabolize the following pesticides: Diazinon, dimethoate, lindane, Thiodan, and Sevin.

Improved evaporated milk. It has been well known that "cooked" flavor which is a deterrent to wider use of evaporated milk as beverage milk can be reduced by using high-temperature-short-time sterilization. However, the improved evaporated milks made by this technique gel rapidly during storage. As a result of EU research it was found that addition of polyphosphates to milk prior to processing markedly reduces the rate of gelation. Practically all U.S. producers of evaporated milk are now testing the commercial applicability of this discovery.

New protein from cow's milk. The existence of a specific "membrane" protein on the surface film of the fat globules in milk has been the subject of speculation and investigation for over 100 years. This protein is essentially homogeneous by electrophoretic measurements, and in view of its composition and properties has been classified as a mucoprotein. Antigenic studies showed it to be immunologically distinct from other milk proteins. The mucoprotein proved to be one of the most potent antigens in milk, and hence a potentially significant factor in milk allergy.



Lake States dairy adjustments. A regional study of dairy farming in the Lake States has indicated the probable overall impacts if all dairy farms were organized in a manner to maximize farmers' net incomes. The area studied includes major portions of Michigan, Minnesota and Wisconsin and smaller portions of Illinois and Iowa. Although fluid milk is supplied to a number of Federal Order Markets, the Chicago Order Market dominates much of the area. A considerable part of the milk production in the region goes into production of manufactured milk products including butter, powder, ice cream, and cheese.

With some increase in demand probable, and under assumptions of improved technology, full utilization of labor, and use of substantial credit, it would be profitable for Lake States farmers to increase production of milk more than 40% by 1965. A substantial increase in milk production would be profitable even with a considerable shift to hog and cattle feeding by farmers in the better soil areas of the region, principally in Illinois, Iowa, and south central Minnesota. In view of a limited demand potential, however, production increases in some parts of the region would need to be offset by decreases in other areas to balance regional supply and demand.

The analysis indicates three profitable adjustments to be of major importance in the Lake States dairy region: (a) Grade A producers generally could provide an increased supply of milk as their competitive position in dairying is strong relative to Grade B producers. This is true even with a reduction of about a third in the historical price premium of fluid eligible milk over milk used for manufactured products. (b) Many Grade B dairy farmers would find it profitable to decrease milk production and increase beef and hog feeding. An increase in these livestock enterprises would also be profitable on some Grade A dairy farms in the Corn Belt type soils of the region. (c) An increase in cow quality and herd size would be profitable on those farms staying in dairy production. On larger, better financed farms, a substantial increase in laborsaving loose housing and milking parlor combination would also be a profitable adjustment.

The largest increase in milk production would be profitable in Michigan where livestock alternatives to the dairy enterprise are limited. This indicated increase in Michigan milk production results in part from a historically "higher priced fluid market" than is the case for other markets within the region. Even though this "higher priced fluid market" is limited, a substantial increase in milk production in Michigan would be profitable.

Indicated reductions in milk production on farms on the Corn Belt type soils of the region are indicative, at least in part, of the strong competitive position of cash crops and non-dairy livestock enterprises. The study suggests that a number of smaller farms in east central Minnesota and west central Wisconsin cannot be organized to provide adequate incomes largely because current land and capital do not provide an adequate base from which to make profitable adjustments. These farms will probably be consolidated into larger units eventually.





## I. FARM RESEARCH

### Dairy Cattle - Breeding Animal Husbandry Research Division, ARS

Problem. Dairy men need information on genetic methods for increasing the efficiency of milk production and modifying milk composition, as well as other economic traits, in order to reduce unit costs and meet the future market demands. Precise information is needed on the relative importance of performance traits, the nature of their inheritance and their response to selection and specific systems of mating. Recently advanced genetic methods, such as those utilizing heterosis and specific and general combining ability, need to be evaluated as procedures for more rapid improvement of milk production or other important traits.

#### USDA PROGRAM

This is a continuing program conducted by geneticists on basic and applied studies of the inheritance of the dairy cow, including experiments designed for evaluating the application of advanced genetic concepts to dairy cattle improvement. The work is in progress at Beltsville, Maryland, and cooperatively with 14 State experiment stations and laboratories in nine foreign countries. Several of the studies contribute to the North Central and Southern regional dairy cattle breeding projects. Cooperation is also carried out with the National Association of Artificial Breeders and with the various dairy cattle breed registry organizations.

The Federal scientific effort devoted to the research in this area totals 18.2 professional man-years. Of this number, 6.0 are devoted to genetics and interrelations of performance traits, 11.0 to selection and systems of breeding, and 1.2 to program leadership.

A grant with the Agricultural Research Center, Tikkurila, Finland, provides for research on the breed differences regarding the antigenic properties of cattle blood, their inheritance in relation to economic characteristics and genetic origin of the breeds. Its duration is for four years, 1961-1964, and involves PL-480 funds with a \$61,804 equivalent in Finnish Finmarks.

Another grant with the Division of Investigaciones Agropecuarias, Ministry of Agriculture, Bogota, Colombia, supports work on the evaluation of the native breed, Costeno Con Cuernos, and Holsteins and Brown Swiss when mated and selected for dairy traits under the hot and humid conditions of Northern Colombia. The duration of the grant is for five years, 1962-1967, and involves PL-480 funds with a \$246,000 equivalent in Colombian pesos.

Two PL-480 projects (also reported in area 6) S3 AH-7, at Sao Paulo, Brazil, and A7 AH-1, at Izatnagar V. P., India, are in effect and are pertinent to this area.

## REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Genetics and Interrelations of Performance Traits

1. The genetics of feed utilization. These studies were undertaken to determine if there are inherited differences in the ability of growing and lactating dairy cattle to utilize feed. The research is being conducted at Beltsville and in cooperation with the Agricultural Experiment Stations of Montana, Utah, and Tennessee. The Tennessee study is a contributing project to the Southern Regional Dairy Cattle Breeding project (S-49).

(a) Genetic ration interactions in feed efficiency and consumption. At Lewisburg, Tennessee, a total of 134 first lactation Jersey cows have completed 305-day production records; 67 on roughage only and 67 on grain plus roughage.

The average mature equivalent fat corrected milk (ME FCM) yield for the roughage group was 9,585 pounds as compared to 11,884 pounds for the roughage plus grain group. The roughage group produced 80.7% as much ME FCM as the roughage plus grain cows. Individual sire groups ranged from 75.7% to 83.4%.

Analyses of these data showed that there were highly significant differences between rations in milk, fat, and FCM production, and in hay and silage consumption. There were also highly significant differences between sires within rations for these measurements. The sire by ration interaction was not significant for either measure of production. The sire by ration interaction was highest in the ME fat yield where 12% of the within ration variation was due to interaction. This, however, was not significant. Analyses concerning the no grain group, where differences between sires in hay and silage consumption were estimated, showed that there were differences between sires in the ability of their daughters to consume roughage. This between sire difference in roughage consumption was in the form of silage and not hay. These preliminary results indicate that there is not an interaction between sires and rations, but there are differences in the ability of daughters of different sires to consume forage.

At Utah, a total of 94 first lactation Holsteins has finished production records, 45 on forage only and 49 on forage plus grain. Six sire groups originated at Huntley, Montana, and 2 sire groups originated at the Utah Station. The average ME FCM yield of the cows fed hay only was 9,559 pounds as compared to 13,425 pounds for the cows fed grain and forage. The hay only cows produced 71.2% as much as their half sibs fed forage and grain. Individual sires varied from 56.9% to 76.8%. The results of a two-way analysis of variance using weighted means indicated significant differences among sires and systems of feeding. The sire by system interaction, however, was not significantly different from zero. These results parallel those from the Jersey data at Lewisburg, Tennessee. The only appreciable difference in the two studies is the higher proportionate production of Jerseys on all



forage ration. It is of significant interest to note that the Jerseys on forage only produced almost the exact amount of FCM as did the Holsteins at Utah (9,585 pounds for Jerseys and 9,559 pounds for Holsteins). These results indicate that Jerseys are more efficient than Holsteins in utilizing forage for milk production. (AH gl-4)

(b) Ration effects on production efficiency. The research in progress at Beltsville, to determine the value of certain feeding regimes in estimating genetic differences in feed efficiency among cows, is being continued. At the present time 25 cows fed at a constant rate have completed 42 lactations. Their average FCM yield was 13,759 pounds with a corresponding feed efficiency of 1.978. The standard deviation of FCM yield and feeding efficiency was 1,323 and 0.27, respectively. Twenty-seven cows fed according to production and maintenance requirements have completed 43 lactations. These cows average 15,084 pounds of FCM and their efficiency of feed utilization was 1.947. The standard deviation of FCM yield and feed efficiency was 2,648 and 0.19, respectively. The difference in yield of 1,325 pounds of the group fed according to requirements was probably due in part to the difference in the average energy intake among the groups. This group received an average of 795 therms of additional energy. There was more variation in FCM yield among cows in this feed group. This would be expected when feeding according to production and maintenance requirements as opposed to feeding at a constant level of energy intake regardless of production and maintenance requirements. The intra class correlations (repeatability) between successive lactations for FCM yield was 0.385 and 0.533 for the constant fed and requirement fed groups, respectively. The repeatability of feed efficiency was 0.283 and 0.449 for the constant fed and requirement fed groups, respectively. The requirement fed group was more persistent and gained more weight during the lactation than the constant fed groups.

(c) Comparisons of ad-libitum feeding and "standard" feeding of dairy cattle. The study on ad libitum feeding of grain, alfalfa hay, and corn silage to 19 second lactation dairy cows is now completed. This study was initiated to study the effects of unlimited feed intake on feed efficiency and milk production. The results of this group were compared against a like number of contemporaries fed at approximately 110% of Morrison's maximum net energy requirements for production and maintenance. The FCM yield, percent SNF, and persistency of production were 13,234 and 13,232; 8.90 and 8.77; 0.70 and 0.68 for the ad libitum and contemporary groups, respectively. These differences were not significant. Body weight, body weight change, therms of estimated net energy consumed, and feed efficiency were 1,486 and 1,409; 248 and 164; 8,671 and 7,397, 1.51 and 1.79 for the ad libitum and contemporary groups, respectively. These differences were significant.

The standard deviation for milk yield was 3,347 and 2,457 for the ad libitum and contemporary groups, respectively. It is apparent that there was considerably greater variation among the cows fed ad libitum. Three of the 19 cows failed to complete a ten-month lactation and milked less than in their first lactation; yet consumed nearly 5% more total energy. Two others,



although completing 10 months, produced less than their first lactation yield, but consumed 32% more total energy. Four cows produced less than their expected second lactation yield. The remaining 10 cows produced from 104 to 133 percent of their expected second lactation yield based on age correction factors.

The ad libitum group consumed 17 percent more total energy on the average than the contemporary group, with a range of 104-151 percent of Morrison's maximum net energy requirements for maintenance and production. Seventy-one percent of the total energy consumed by the ad libitum group was in the form of concentrates. As a result of the additional energy consumed, and because of the variation in response for yield, the ad libitum group gained more weight and was less efficient than the contemporary group. The standard deviation for therms of energy consumed was 1,304 and 448 therms for the ad libitum and contemporary groups, respectively. This difference was significant and indicates the variation among cows in their ability to consume unlimited quantities of feed.

In comparing the standard deviations for each of the production traits studied, from the first to second lactation of the ad libitum group, there was a significant increase in all except body weight. During the first lactation, these cows were fed according to requirements. The standard deviation of FCM yield, persistency, total therms of energy consumed; body weight changes, and feed efficiency increased by 1,715 pounds, 0.04%, 686 therms, 36 pounds and 0.08 therms of energy per pound of FCM yield, respectively. When the standard deviations for the same traits were compared between the first and second lactations of the contemporary groups, there were no significant changes. These results are a further indication of the variation among cows in their response to unlimited feed intake.

The ad libitum cows were less efficient in their second lactation (1.51) than in the first lactation (1.69). Previous work has shown efficiencies to increase in successive lactations, which occurred in the contemporary group (1.69 to 1.79) from first to second lactation.

Since there was an increase in the amount of energy consumed with no difference in FCM yield among the groups during second lactations, a decrease in gross efficiency would be expected. The increase in the variation in body weight change is also a reflection of the additional energy consumed. Once the individual requirement for maintenance and production was met, the additional energy was being utilized in laying down additional flesh.

Several rather important factors have evolved as a result of these studies. The first is the apparent variation among cows in both their response to ad libitum feeding and the variation in cattle in their ability to consume large quantities of feed with varying proportions of concentrates to roughages. Secondly, it would appear that ad libitum feeding may be a useful tool in the genetic aspects of feed utilization in dairy cattle, because of the magnitude of the between cow differences in their response to ad libitum



feeding. Third, it points out rather clearly that appetite "per se", although important, is not the only limiting factor in milk production. (AH gl-4)

2. Effects of lactation stage and number on type ratings. A group of 200 first lactation Holstein cows, in three different Ohio herds, were evaluated for type on a complete breakdown basis including ten categories. Evaluations were made independently and simultaneously by two persons, three months after calving, eight months after calving, and at approximately the middle of the dry period following the first lactation. For each evaluation period, the scores of the two classifiers were averaged. Data were analyzed by the analysis of variance technique to determine the relative importance of stage of lactation, and correlation values were used to estimate repeatabilities.

Categories significantly affected by stage of lactation were middle and loin, rump and thigh, udder attachment, and breed character. As lactation progressed, type scores for these categories increased. For the category dairy character, there was a gradual decrease in type score in all herds as lactation progressed. This decrease, however, was not significant at the .05 level of probability.

Correlations between type score and milk production were computed for each of the periods (3 months after calving, 8 months after calving and dry). The type categories of middle and loin, rump and thigh, and breed character differed significantly between the periods. These results indicate the errors involved when type ratings are made on cows in different stages of lactation. Other correlations were computed as follows: First lactation type vs. first lactation production 0.024; first lactation type vs. mature production 0.085; mature type vs. mature production 0.155; and 3 months after calving type vs. 3 months after calving production 0.147. These correlations are small and have little value in predicting milk production. (AH gl-2)

3. Relation between body form and milk production. Data collected during the years 1949 through 1961 from the Grand Rapids, Morris and Rosemount Experiment Station dairy herds were analyzed to study the relationships between certain body measurements and milk yield. Estimates were obtained from 157 daughter-dam pairs and from paternal sister data representing from 371 to 452 progeny of 71 sires. Body measurements studied were weight, height at withers, chest depth, body length, heart girth and paunch girth. Measurements were taken at 3 months, 6 months, 12 months, and 18 months of age, and at 3 months after first calving.

Most of the combined estimates of the genetic correlations between body measurements and production were positive and on the order of .10 to .40. However, with the exception of the correlation between 12-month weight and milk yield, none of the estimates were significantly different from zero. The genetic correlation between 12-month weight and milk yield was estimated to be  $.434 \pm .185$ .

Analysis of the body measurement data suggests that no genetic antagonism exists between milk yield and measurements of body size. Dairy men interested in feeding dairy steers can expect their selection for milk yield to result in no decrease in the gains made by offspring of their better yielding cows. (AH gl-1)

4. Early sire evaluation by the use of udder palpation and body weights. At Ohio, data on 55 sires which had production records on 1,216 daughters were used to determine the accuracy of an early sire evaluation based upon the udder palpation information available on his five-month old daughters.

Future production of the daughters was predicted from the udder palpation information by the use of five prediction equations developed in the NC-2 and S-3 interregional bulletin (1960). The resulting predicted production was averaged by year of daughter's birth for each sire having four or more palpated daughters born in one year. Thus, 149 predicted daughter averages were available for comparison with the daughters' actual performances.

The predicted daughter averages were correlated with the actual daughter averages on a within herd and within year basis. The correlations for two of the prediction equations were significantly different from zero (at 1 percent level). However, analysis of the various components of the indexes indicates that the majority of this association is a result of the inclusion of the herd average and dams production in prediction indexes. It is concluded that udder palpation information is not a reliable method of obtaining an early sire evaluation. (AH gl-3)

5. Meat production from beef, dual purpose and dairy steers. This study was initiated in cooperation with the Beef Cattle Research Branch to determine the relative merits of various breeds of cattle and different management systems in the production of meat.

The first relication has been completed of this experiment in which Holstein-Friesian, Milking Shorthorn, Jersey, and Aberdeen-Angus steers are being fed during the first six months of life (phase 1) either on a high or low plane of nutrition and then placed on 3 types of rations per group until slaughter (phase 2). Average 180-day weights were 440 and 221 pounds on the high and low planes, respectively. Holsteins gained most rapidly on all rations in both phases followed by Milking Shorthorns, Angus, and Jerseys in that order. Holstein-Friesians gained proportionately faster and the Milking Shorthorns slower on the low nutritional plane during the first six months. Breed X ration interactions in the second phase and at slaughter were significant only for daily gain, thickness of fat over the rib eye and percent internal fat. Animals fed at the lower level during the first six months gained more rapidly and more efficiently during the second phase. Carryover effects of first six-month treatment on carcass grade and composition were small and non-significant. The lean, after cooking to an internal temperature of 140°F. (60°C.), was significantly more tender by both Warner-Bratzler shear and taste panel for those on the high plane the first six months. Tenderness



and palatability of the meat, fat content, and lean content were related to the feeding regimes during the second phase. Those receiving more concentrates had more fat, less lean, and more tender and palatable lean. In fatness of carcasses the breeds ranked Aberdeen-Angus, Milking Shorthorn, Jersey, and Holstein-Friesian. The Angus ranked highest and Milking Shorthorn lowest in tenderness. Ration differences in efficiency expressed as live weight gain or pounds of lean produced per therm of energy consumed were small. Ration effects were significant for pounds of fat produced with the high level nutrient rations producing the most fat. Breeds were significantly different in efficiency of both gain and lean production and ranked in the order of Holstein-Friesian, Milking Shorthorn, Aberdeen-Angus and Jersey. The Aberdeen-Angus was the most efficient in fat production. (AH d3-6)

6. Meat production from dairy steers. This study was designed to evaluate the variation among sire groups in meat production characteristics when selection is made for milk yield. It will also measure the genetic relationship between meat and milk production. The project is cooperative with the Experiment Stations of Pennsylvania and West Virginia. At Pennsylvania State University, a comparison was made concerning growth and carcass evaluation on both Holstein steers and bulls. Groups of 15 bulls and 15 steers were fed out to weights of 800 and 1,000 pounds. The average daily gain of bulls was significantly greater than steers from birth to slaughter at both 800 and 1,000 pounds ending live-weight. The days required to reach slaughter weights were significantly less for bulls than for steers. The steers, however, had higher dressing percentages than bulls at both slaughter weights. The bulls were larger in rib eye area and had superior percent of desired cuts than did the steers. Steers were superior in percent of hind quarters as compared to bulls.

7. The genetics of blood antigens in dairy cattle.

(a) Blood group segregation and fertility effects. At the the Ohio laboratory, a study was made of the possible effects on fertility of blood group segregation. Five bovine blood groups (A, F-V, L, S, and Z) were evaluated in Holstein cattle to determine whether blood group incompatibility existed. Incompatibility was based on the difference between observed and expected allelic segregation to female offspring. The only system deviating significantly from the expected allelic segregation was the F-V system. This deviation ( $P = 0.005$ ) favored F/F over F/V type offspring from the mating type F/V x F/F (615 to 515, respectively). Preliminary evidence indicated that postnatal removal prior to typing was not a contributing factor. Comparisons of the percent delayed returns (28 days) for total pregnancies and calves born for total services between the mating types F/V x F/F and F/F x F/F were 20.4 to 17.7 percent and 50.7 to 48.5 percent, for 642 and 706 pregnancies and 1,197 and 1,017 services, respectively. These differences were not significant although a trend of a greater delayed return rate may have been indicated with the former mating type.

No discrepancy was found in allelic segregation of the F/V x F/V mating types.

(b) Transferrin types and their relation to fertility and production.

Another study at Ohio, associated bovine transferrin types with fertility and production. Samples of bovine blood from 1,116 animals were used to determine transferrin (Beta-globulin) types. Disc electrophoresis procedures were developed which would accommodate bovine bloods. Genes frequencies for transferrin types among 456 Holstein females in one Ohio NC-2 Project herd were TrA-28.8%, TrD-62.5%, and TrE-8.7%. Parental comparisons indicated that transferrins were controlled by a three allele system without dominance. Age of the animal (one day to five months) had no detectable influence on transferrin type. The frequency of transferrin types appeared similar for both sexes. Analysis of 520 sire-dam-daughter combinations and 32 supposedly identical twin pairs indicated that the transferrin data are very valuable in supplementing blood antigen data for identification purposes. Information on several hundred matings indicated no easily detected association between transferrins and fertility. A significant association between transferrin type and milk production was demonstrated (215 cows). Animals without TrE were better producers than those with TrE.

(c) Blood antigen studies in Finland. The PL-480 project in Finland on bovine blood antigens is in its third year. The Finnish laboratory has produced 30 blood typing reagents and is now practically self-sufficient with regard to these typing fluids. In the development of reagents at least one new antigenic factor, SF<sub>3</sub>, was discovered. This factor may be controlled by a previously undiscovered locus. It seems to be inherited independently of nine of the eleven known loci.

Further studies of blood groups in relation to a recessive gene for hairlessness have been made. The previous indication of association between the gene for hairlessness and blood factor L<sup>1</sup> was not confirmed. It now appears that the gene for hairlessness is not associated with blood groups at nine loci which have been studied. Furthermore, studies in Finland failed to indicate any association between the dominant gene for polledness and blood groups.

A survey was made to determine the frequency of incorrect parentage. A random sample of 588 young bulls revealed that 2.38% had incorrect pedigrees. In a similar sample of 718 young daughters of AI sires 5.32% had incorrect pedigrees. The combined figure is 3.98%. (AH gl-6) (PL-480-E8-AH-1)

8. Genetics of milk constituents. The national cooperative effort to obtain data on milk, milk fat, solids-not-fat (SNF) and protein production of individual cows is continuing. Plans are being made for a preliminary analysis of data to study genetic and non-genetic influences which affect milk composition. Several thousand lactation records are available from all four regions of the U. S. and they are to be sent to Virginia Polytechnic Institute for analysis. Expectations are that for the Holstein breed



at least, there will be over 5,000 lactation records.

(a) Evaluation of testing methods. Interest in testing methods, particularly for protein, has heightened considerably during the past year. The dye-binding methods for protein determination are widely used. They are good methods under laboratory conditions where the variables involved can be carefully controlled. Considerable research needs to be done, however, before a single standard method can be recommended for widespread use in protein testing, as the Babcock test is used for milk-fat testing. Dairy Cattle Research Branch personnel are cooperating with the American Dairy Science Association and the Association of Official Agricultural Chemists to find out how reproducible the dye-binding methods are and to develop a standard method. In the first attempt at collaborative testing, twenty samples of dry milk powder were sent out from Beltsville in the fall of 1962. Collaborating laboratories were asked to test the samples (all laboratories received the same samples) by the standard Kjeldahl method for protein as well as their dye-binding method. Eight laboratories tested the samples by the Kjeldahl method. The average differences from expected were .23 and .24 percent for two of the laboratories (sign of difference not considered). The averages for the other six laboratories were between .046 and .075. The range of values reported for any one sample by these six laboratories averaged .21.

Six laboratories tested the samples by the Orange G dye-binding method. The range of values reported for any one sample averaged 1.02 percent. One laboratory reported results considerably higher than the others and when its results were excluded the average dropped to .42 percent. Four laboratories used the Amido Black dye-binding method with the range of values reported for any one sample averaging .65 percent. Deviations from expected were not calculated since the dye-binding values were taken from curves established with whole milk and there was a difference in the dye-binding capacity of the powder as compared to fresh milk.

One of the problems encountered in the dye-binding methods is variation in dye purity. During the past year, the Color Certification Branch, Division of Color and Cosmetics, Food and Drug Administration (FDA) has cooperated by assaying dye samples. This work revealed considerable variation in the pure dye content of different lots of dye. Three different experiments at Beltsville have indicated that the practice of standardizing dye solutions by making them up to the same optical density may lead to errors. In the most recent and extensive study, solutions were made up from four different batches of Orange G dye. The FDA analysis showed the preparations to contain 96.0, 95.9, 91.3 and 96.2 percent pure dye, respectively. The four solutions were prepared to give the same optical density. Careful comparisons were made with each solution being read several times on three different occasions over a 2-day period. Milk samples for each of ten cows were tested in triplicate with each of the four dye solutions. Twelve tests were done on each cow's milk for a total of 120 tests. Analysis of variance showed that differences due to cows and dyes were highly significant. There was no evidence

of cow x dye interaction. The overall mean protein percentages for the four dye solutions were 3.39, 3.42, 3.46, and 3.50, respectively. This variation could not be explained on the basis of percent pure dye since the solutions giving mean protein percentages of 3.46 and 3.50 had 91.3 and 96.2 percent pure dye, respectively, while 3.39 and 3.50 were the mean protein values for the solutions with 96.0 and 96.2 percent pure dye. The conclusion is that specific unknown impurities in the dye are responsible for the observed differences. More work is needed to find out what these impurities are and the extent to which differing amounts of them affect optical density.

(1) Interrelationship of milk constituents. At Beltsville, records have been summarized on 147 cows that have completed lactations in which their milk was tested monthly for fat, SNF and protein. Of these, 124 were Holstein, 11 were Brown Swiss-Holstein crossbreds and 12 were Ayrshire-Holstein crossbreds. There were 183 lactation records. These records averaged 15,477 pounds of milk, 4.09% fat, 633 pounds of fat, 8.86% SNF, 1,371 pounds of SNF, 3.13% protein and 485 pounds of protein (2X 305 ME records). The following correlations were calculated from lactation averages on a within sire basis: milk and fat % -0.27, milk and SNF % -0.03, milk and protein % -0.18, fat % and SNF % 0.50, fat % and protein % 0.34, and SNF % and protein % .31. The correlation between SNF % and protein % is considerably lower than expected in comparison with reports from other workers.

Correlations among these same variables were calculated for each of nine sire groups (7 to 17 daughters per sire with 9 to 21 records per sire group). Although these are preliminary analyses on relatively small numbers, the results show different relationships of milk constituents between bulls. Of the 54 correlations calculated, only 11 were significantly different from zero. The significant sire group correlations were: milk and fat % -0.50 and -0.50; milk and SNF % 0.44 and -0.71; fat % and SNF % 0.48, 0.70, 0.71, 0.74 and 0.86; fat % and protein % 0.48; and SNF % and protein % 0.63. A further preliminary indication of variation between sire groups is seen in the sire group means for fat, SNF and protein %. One group of 10 daughters of Holstein cows by a Brown Swiss bull averaged 4.57% fat, 8.90% SNF and 3.09% protein. The sire group with the lowest average fat % (3.79) had an average SNF % of 8.77 and an average protein % of 3.13. This was a purebred Holstein group of 13 daughters with 16 records.

At Michigan State University, the composition of milk has been measured for about 10,000 completed lactations. Preliminary analysis shows almost perfect correspondence between pounds of milk and pounds of protein, lactose and minerals produced in completed lactations. Variation in percent solids of milk excluding fat appears similar to the way and amount percent fat varies with the age of the cow and with the amount of milk produced. Genetic relationships between milk and its components are being examined.

(b) Genetically controlled protein variants. Additional studies have confirmed the previously reported suspicion that genetically controlled variation occurs in  $\alpha_s$ -casein. This work is done in cooperation with the



Eastern Utilization Research and Development Division. Caseins from 1,378 individual cows were studied and three major  $\alpha_s$ -casein variants were identified by starch-gel-urea electrophoresis. They are designated A, B, and C in order of decreasing mobility. Family studies indicate that an autosomal series of three genes,  $\alpha_s$ -C<sub>n</sub><sup>A</sup>,  $\alpha_s$ -C<sub>n</sub><sup>B</sup>, and  $\alpha_s$ -C<sub>n</sub><sup>C</sup>, controls the variation in  $\alpha_s$ -casein. Each gene causes the appearance of a particular form of  $\alpha_s$ -casein and there is no dominance.  $\alpha_s$ -casein B is most common. Seventy-two percent of all animals sampled had only B in their milk. The other percentages were: A only, 0.1; A and B, 5.9; A and C, 0.4; B and C, 18.6; and C only, 2.5. Ninety-eight Ayrshires were sampled and all had B only. All three variants, A, B and C, were found among the 542 Holsteins sampled. Only B and C have been found so far in Brown Swiss, Guernsey and Jersey cattle.  $\alpha_s$ -casein C is more common in Guernseys and Jerseys than in any of the other breeds.

$\beta_s$ -casein types were also determined on the 1,378 milk samples. The three types, A, B, and C were found and family studies confirmed.

Further studies of additional animals are planned to determine if there are any associations between these polymorphisms and other traits.

(c) Flavor differences in milk from individual cows. Milk samples for organoleptic evaluation were taken monthly throughout lactation from 74 cows. Samples were split, pasteurized and stored at 40°F. until a panel of 10 judges at the Eastern Utilization Research and Development Division evaluated them for flavor. One subsample was judged by the taste panel on the morning following collection (fresh samples), and the other was judged after 7 days of storage (stored samples).

Least squares analysis indicated that there were significant differences associated with season for cooked, feed, oxidized, salty and total score flavors. Stored samples showed differences in cooked, feed, flat, oxidized, salty and total score. Milk produced during April-July period had the best flavor. There were significant changes associated with stage of lactation in the following: feed, oxidized, salty and total score in fresh samples and feed, flat and salty in the stored samples. Higher quality milk was produced during the first half of lactation. The analysis also indicated important differences in flavor of milk produced by individual cows and between groups of half-sibs.

Within cow correlation analysis showed that pounds of milk, percent fat, percent solids-not-fat, quantity of feed consumed, air temperature and relative humidity had little relation to total score or specific criticisms of either the fresh or stored samples. The within-cow correlation between fresh and stored total score was only 0.19. (AH gl-5)

## B. Selection and Systems of Breeding

1. Comparisons of inbreeding and outbreeding. This research was undertaken to determine the effects of inbreeding, outbreeding, and interline



crossing on production and other economic characteristics of dairy cattle. It is conducted cooperatively with the Wisconsin Agricultural Experiment Station and is a contributing study to the North Central Regional Dairy Cattle Breeding Project. The development of crosses among six inbred lines of Holstein-Friesian cattle and maintenance of controls have been continued. The effects of mating systems on production traits were evaluated. Analysis of the control group showed non-significant variations among years and among season of calving. Therefore, all analyses were performed without consideration of yearly and seasonal effects.

(a) Interaction effects. Analysis of all traits between different systems of mating for each line of sire and that between lines for each mating system indicated the existence of system X line interaction, i.e., the differences among mating systems were not consistent from line to line. In general, inbreeding decreased the mean value of all traits except fat percent and age at first calving. The superiority of the two-line cross and three-line cross groups over the inbred groups can be interpreted as an expression of heterosis in these traits.

(b) Regression estimates of inbreeding effects. The data were divided into two sets. Set one consisted of outbreds and all inbreds. Set two included the same inbred animals and the two-way linecrosses. Within-line estimates of inbreeding effects on milk production were -54.9 and -67.1 pounds of milk per each 1% of inbreeding for the two sets of data, respectively. Corresponding estimates for butterfat were -1.7 and 2.7 pounds. Fat percent and age at first calving showed little effect of inbreeding. Body weight at first calving was decreased by 2.2 pounds in set one and 3.2 pounds in set two for each 1% of inbreeding.

(c) Changes in variance and covariance component estimates under different systems of mating. The total variance, between-line variance and within line variance components tended to increase with inbreeding and decrease with crossing in most traits. However, the within-line variance for fat percent indicated a slight decrease with inbreeding. The covariance between different traits on a between-line basis showed a tendency to increase with inbreeding. The daughter-dam covariance components estimated on a within-line basis in which daughter and dam were produced under the same or different mating system, showed an increase in milk and fat but a decrease in fat test with inbreeding. Data from crosses of the inbred lines, when compared with the estimates for the outbred group, yielded larger estimates of the covariance components for milk and fat with little change in the values for fat test. The biological interpretations of such changes are obscure because of the sampling error of the estimates, the unknown genetic situation of the traits under study, the presence of dominance, and possibly other reasons.

(d) Genetic variation in body size at birth. The variation of body weight and measurements of 559 single-born Holstein calves of both sexes at birth was studied. Least-squares estimates of sex and parity effects were



used for adjustment of all measurements to a first-parity-female-calf basis. The mean value of each trait decreased and the total variance increased with inbreeding. The changes of the within-line variances were not consistent, but the between-line variances tended to increase in the inbred groups. When outbred calves and inbred calves from outbred dams were compared, inbreeding depression was highly significant in body weight, heart girth, hip width and shinbone circumference. Significant heterosis was shown in all measurements except shinbone circumference when inbreds and two-line crosses (both from inbred dams) were compared. A regression analysis of data from all outbred and inbred calves indicated significantly heterogeneous effects of inbreeding among sire lines in chest depth, hip width, head length and width. The intra-line regression was  $-0.24 \pm 0.05$  pounds and  $-0.06 \pm 0.01$  cm for each increase of 1% inbreeding in birth weight and heart girth, respectively.

(e) Factors affecting multiple ovulations. A total of 3,076 ovulations determined by palpation in 728 animals covering six sire lines, two systems of mating (outbreds and inbreds, with inbreeding coefficient not exceeding 40%) and three parities were analyzed. The study was limited to observations during 90 days from the day of calving in cows or from 12 months of age in heifers. Ovulations which occurred in service periods characterized by cystic ovaries ("cystic" service periods) were studied separately. The incidence of multiple ovulations in noncystic and cystic service periods was 4.2% and 12.9%, respectively. Analysis of ovulations in noncystic service periods by the method of least squares showed that outbreds had a significantly higher percentage of multiple ovulations than inbreds (5.2% vs. 3.2%  $P < 0.05$ ). There was a significant increase in the incidence of multiple ovulations with parity (from 2.9% in heifers to 5.9% following the second calf  $P < 0.05$ ). The influence of sire line was nonsignificant. Multiple ovulations were not significantly associated with expression of heat (silent or expressed) or with interval since calving in cows or since 12 months of age in heifers. Intracow correlation of multiple ovulations within line, system, parity and season was found to be 0.074 ( $P < 0.01$ ). (AH g2-5)

2. The relative importance of general and specific combining ability in breeding dairy cattle. These studies were undertaken to determine the relative importance of general and specific combining ability between lines of dairy cattle within the same breed. They are designed to study the genetic methods needed for utilizing the non-additive genetic variance which may be present in the economic characteristics of dairy cattle production. Projects are cooperative with the Minnesota and Ohio Agricultural Experiment Stations and are contributing projects to the North Central Regional Dairy Cattle Breeding Project.

(a) Line development progress. At Minnesota, progress in the line development program at Rosemount was checked by recomputing the average relationship between milking age animals in the line. This relationship is now 8.1%. The Southern Experiment Station at Waseca was brought into the project by transferring some of the Rosemount animals to that station.



Eventually, about 50 milking linebred animals will be located at Waseca with a like number at Rosemount. All matings in the Rosemount-Waseca herds are to sires closely related to four descendants of Wisconsin Admiral Burke Lad. Plans are developed to increase the relationship between animals at a more rapid rate. The line development project at Morris and Crookston has proceeded as planned.

At Ohio, the line development program is progressing according to plan. Four herds are sufficiently linebred to permit between-line crossing. Two other herds are approaching the point where they can be crossed. In order to make an objective evaluation of genetic differences between lines, the frequency of alleles for blood types were evaluated for three of the lines. It is evident that lines do differ in this respect. In the A system, the frequency of the A allele was .439, .130 and .106 for the Darkmaster, BDI and Berea lines, respectively. Corresponding frequencies for the L allele in the L system were .521, .136 and .053. The frequency of occurrence of allele in 4 of the 10 antigen systems was found to be different among the lines.

(b) Inbreeding effects on production. A study was made to evaluate the low levels of inbreeding as they influence production within the line developing herds. Within-year-season, herd, sire regression analyses independent of age of Holstein cows in each of their first four lactations were derived from the data. Regression analyses for the first, second, third, and fourth lactations involved 1,233, 784, 480, and 263 within subclass degrees of freedom, respectively. The regressions of first lactation 2X, 305-day, actual milk and butterfat yields on 1% of inbreeding were  $-41.3 \pm 9.2$  and  $-1.24 \pm 0.33$  pounds, respectively. When heart girth was included in the preceding analyses as a third independent variable, the regression statistics remained the same. The regressions of second lactation milk and butterfat yields on inbreeding independent of age were  $-42.9$  and  $19.3$  and  $-1.19 \pm 0.54$  pounds, respectively. For the third and fourth lactations, the regressions were within the magnitude of their standard errors of zero; for the fourth lactation they were positive. First lactation data of cows that had four lactations yielded regressions which were close to zero. These results suggest that decreasing effects of inbreeding as shown by other studies are not due to maturity of cows but rather are due to selection and culling. (AH g2-22)

3. The influence of parental relationship on the genetic merit of dairy cows and sires. This research was undertaken to determine the relative merits of line-breeding, outcrossing and crossbreeding using progeny tested bulls of high merit as service sires. Mating plans were continued for the foundation cows and for first and second generation animals in each of the various mating systems. The present herd consists of 78 outcrosses, 69 linebreds, 83 crossbred and 29 foundation females. The actual first lactation averages for the various groups are as follows: twenty-seven linebreds averaged 11,394 lb. milk, 3.94% BF., 449 lbs. BF., ave. age of 25.5 mos.; twenty-seven outcrosses averaged 10,250 lbs. milk, 4.25% BF., 436 lbs. BF., ave. age of 25.8 mos.; eight Ayrshire-Holstein crosses averaged 10,702 lbs.

milk, 4.24% BF., 454 lb. BF., ave. age of 25.3 mos.; and twelve Brown Swiss-Holstein crosses averaged 8,598 lbs. milk, 4.37% BF., 376 lb. BF., ave. age of 25.1 mos.

(a) Growth and feed efficiency among groups. Heifers from each mating system were placed on a standardized ration of free choice alfalfa hay and 3 pounds of grain per day from 12 to 16 months of age. Feed efficiency, rate of gain and hay consumption studies were made on 165 heifers. These represented the 61 outcross heifers by 6 sires, 50 linebred heifers by 5 sires; 24 A X H crosses by 2 sires; 25 S X H crosses by 2 sires, and 5 S X A H crosses by 1 sire. Analysis of variances indicated significant differences among groups and among sires within groups for therms of energy consumed in hay and average daily gain over the 120-day experimental period. The therms of energy consumed in hay were 1,154, 1,115, 1,078, 1,178 and 1,069 for outcrosses, linebreds, A X H crosses, S X H crosses and S X A X H crosses, respectively. The average daily gain (ADG) was 1.70, 1.66, 1.56, 1.72, and 1.65 for the outcrosses, linebreds, A X H crosses, S X H crosses, and S X A X H crosses, respectively. There were no differences among the groups or among sire within groups in efficiencies of feed utilization. It appears that all groups utilized the energy intake at the same relative efficiency.

The second objective of these trials was to determine if there was any relationship between total gain, rate of gain and efficiency of gain during this 120-day period and FCM yields and efficiency of FCM yield during the first lactation. To date, 74 females have completed their first lactation; 27 outcrosses by 3 sires, 24 linebreds by 3 sires; 11 A X H crosses and 12 S X H crosses by one sire each. None of the correlations were significant. The general trend in all groups except the linebreds was negative. (AH g2-24)

(b) Cow loaning program. After the Beltsville cows finish a required number of lactations, they are loaned out to cooperating dairymen in the area. The purpose of this program is to evaluate the performance of the various breeding groups in other environments. The cooperators also mate 10 of their own cows to young Beltsville bulls from each of the three breeding groups to determine the value of bulls produced by the various systems. To date, a total of 112 cows have been loaned. These cows averaged 15,085 pounds of milk, 3.98% and 601 pounds of butterfat at Beltsville. Sixty-eight cows with completed records in cooperator herds averaged 2,802 pounds of milk, 136 pounds of fat and 0.20% test less than their Beltsville records. A stablemate comparison of Beltsville cows vs. cooperator-owned cows indicated an advantage for the Beltsville cows of 168 pounds of milk and 10 pounds of butterfat. This small advantage is only partially indicative of genetic superiority. Adjustment to new environment had a depressing effect on production. (AH g2-24)

#### 4. The use of progeny tested sires and sons of progeny tested sires.

(a) Production-longevity relationships. Production and age data on



3,879 daughters of 123 Holstein sires were used to study the relationship between first-lactation milk and milk-fat production and longevity in 79 herds. They were divided into six groups by type of ownership.

The average age at the start of the first lactation was 32.4 months, and at the start of the last complete lactation (final age) was 61.1 months. Linear regressions of final age on first-lactation production were 0.071 months/lbs. milk fat and 0.024 months/10 lbs. of milk. Corresponding correlation coefficients were 0.188 and 0.179, respectively. Differences between the owner group regression coefficients were not statistically significant. Individual sire regressions of final age on milk production varied from  $-0.089 +$  to  $+0.129/10$  lb. of milk and from  $-0.253$  to  $+0.346$ /lb. milk fat. The differences between the sire regression coefficients were significant. Regression coefficients at different levels of milk production within owner groups varied widely, but differences between these regressions were statistically significant in only one group.

The regression and correlation coefficients were small but highly significant. There seems to be little doubt that on the average in these data, the higher-producing first-lactation cows had a somewhat longer productive life.

(b) Means and variances for production traits in different lactations of the same cows. The first four lactations of 1,228 cows and 1,187 of their dams were used to study the means and variances of days in milk, age at start of lactation, pounds of milk, pounds of milk fat and percent of milk fat. The differences between lactation means were significant for all five characteristics for both daughters and dams even after adjustments were made for age. The error variances in the four lactations were found to be significantly different for all five characteristics in the daughter data and for all characteristics except pounds of milk in the dam data. If more extensive studies bear out these results, then the practice of pooling production data over different lactations of the same cow could lead to erroneous interpretations.

(c) Age of calving effects on production. The influence of age at first calving and calving interval on production per day of life and total lifetime production were studied. Lifetime production records of 450 Holstein cows from the combined USDA - Utah Agricultural Experiment Station herd were used. The influence of calving interval was obtained from the records of 368 cows that had calved more than once. Cows freshening at 25 months of age had the highest production of milk and butterfat per day of life, with production per day decreasing as the age at freshening increased past 25 months. Highest total lifetime production was made by cows freshening at 30 months, followed by the freshening at 27 and 28 months of age.

A calving interval between 12 and 13 months resulted in highest production per day of life. However, cows with calving intervals between 13 and 15 months tended to stay in the herd longer and achieved a higher lifetime production. Correlations between pounds of milk produced per day of life



since two years of age and age at first freshening, calving interval, age cow left herd, percent days in production since two years of age and since first calving were  $-.34$ ,  $-.11$ ,  $.17$ ,  $.50$  and  $.24$ , respectively. Correlations for production of butterfat per day of life were similar. (AH g2-25)

5. Usefulness of heterosis resulting from interbreed matings. These studies are concerned with the theoretical and practical aspects of heterosis resulting from interbreed matings among dairy breeds. Projects are in progress at Beltsville and in cooperation with the Illinois and Indiana Agricultural Experiment Stations. The Beltsville study is a contributing project to the Southern Regional Dairy Cattle Breeding Project and the cooperating projects contribute to the North Central Regional Dairy Cattle Breeding Project. Most of the semen services are supplied through the cooperation of the National Association of Artificial Breeders.

(a) Genetic correlations between dairy and beef traits. Data from the Indiana project were analyzed to determine phenotypic and genetic correlations and heritability estimates for dairy and beef traits in purebred Red Danes, Milking Shorthorns and Red Polls; all possible two-breed crosses; and certain three-breed crosses. In general, the phenotypic correlations of the milk traits with feed efficiency and rate of gain were small and mostly positive. Milk and fat yield exhibited slightly negative correlations with percent lean cuts and positive correlations with carcass grade. Percent fat in the milk was correlated negatively with carcass grade and positively with percent lean cuts. None of these correlations were significantly different from zero. The genetic correlations were larger in absolute value and had the same sign as the phenotypic correlations. The confidence limits associated with them were extremely large so the genetic correlations could only be crude estimates of the true genetic correlations. However, they did indicate that some genes could be effective in both the dairy and beef traits.

Low negative correlations were found between the slaughter traits (carcass grade and percent lean cuts) and the growth traits (feed efficiency and rate of gain). Based on the phenotypic correlations from this study, selection could be made for either beef or dairy traits without serious determinable effects on the other. Also, selection for both milk and beef traits could be made simultaneously with only a slight reduction in the selection differential for each trait. (AH g2-23)

(b) Effects of crossbreeding on feed efficiency. Further analyses of feed efficiency data on Red Poll, Red Dane and Milking Shorthorn cattle and their two- and three-breed crosses in the Indiana project have been made. The purebred steers required 8.51 lb. feed/lb. gain (4.63 lbs. was in excess of maintenance requirements) and gained 2.02 lbs. daily. The two-breed crosses required 8.33 lb. feed/lb. gain (4.58 lb. above maintenance) and gained 2.10 lbs. daily. The three-breed crosses required 7.74 lb. feed/lb. gain (4.12 lb. above maintenance) and gained 2.21 lbs. daily. The major genetic effect appeared to be general combining ability or an additive genetic effect. Least squares estimates indicate the crossbreds gained faster



and more efficiently than purebreds. None of the differences were of statistical significance at the 5% level of probability although a few approached that level. However, the crossbred-purebred comparisons indicate the Red Dane-Milking Shorthorn crosses showed more evidence of heterosis than any of the other crosses. From these results, it appears the beef producing capacity of the male offspring may be enhanced to some extent by crossbreeding. (AH g2-23)

(c) Effects of crossbreeding on growth rate. In the experiment at the University of Illinois, involving crisscrossing between Guernseys and Holsteins, nine different measures of skeletal dimensions and weight were recorded at 9 different ages (from 3-60 months of age) to compare the growth rate of second generation or 3/4 bred animals to the purebred parent breeds. The means for the crosses exceeded the parental mean in 79 of the 81 cases indicating heterosis for growth rate with only 25% blood from a second breed. The differences favored the crosses to the greatest extent up through 24 months of age. The differences at later ages were small. For example, crossbreds exceeded the parental mean by an average of 4.7% in body weight up through 24 months but only 2.8% from 30 to 60 months. The 3/4 Holsteins were slightly larger than the 3/4 Guernseys but neither of the crossbred groups were as large as the purebred Holsteins. The crossbreds averaged 2 1/2% in wither height and 9% less in body weight than the Holsteins.

Preliminary data on first generation crosses among Ayrshires, Brown Swiss and Holsteins in the project at Beltsville, also indicate a small degree of heterosis for growth. The crossbreds have exceeded the expected or parental mean by 2 to 4% in body weight at 3, 6, 12, 18 and 24 months of age, and 1 to 2% in height at withers, depth of forechest, forechest girth and body length, at 6, 12 and 18 months of age. None of the crossbred groups have exceeded the growth rate of the purebred Holsteins except the Brown Swiss-Holstein crosses which were slightly heavier at 18 and 24 months of age. (AH g2-23)

(d) Effects of changes in body weight during lactation on production of purebred and crossbred cows. Body weight data on the foundation, first, second and third generation purebred Holsteins, reciprocal crossbreds and purebred Guernseys in the Illinois project were analyzed to determine the changes in body weight during the first and second lactation periods and the relationships of changes in body weight to milk and fat production. Data on 239 first and 157 second lactation animals were used in the analysis.

Partial regressions of weight on month of lactation indicated that cows of a constant age gained approximately 14.8 lb./month during first lactation and 15.0 lb./month during second lactation. The gains in the different months of lactation did not differ significantly.

The partial regressions of weight on age for a constant month of lactation indicated purebred Holsteins increased approximately 12.6 lb. for each one month in age during both first and second lactation. The corresponding



increases for Guernseys, crosses by Holstein sires and crosses by Guernsey sires were 7.8, 7.2 and 9.5, respectively.

With calving age and weight 30 days after calving held constant, the partial regressions of milk yield on gain in body weight during first lactation indicated that cows produce 10.9 lb. less milk for each one pound gain in body weight and 12.6 lb. less in second lactation. Fat yield was decreased by .41 and .48 lb. for each pound of gain during first and second lactation, respectively. Age at calving and body weight 30 days after calving were not closely associated with milk and milk fat yield during the first and second lactations. Body weight from 30 days after the beginning of first lactation to the end of the second lactation increased 23.3, 26.1, 27.4, 29.6 and 26.4% for purebred Holsteins, purebred Guernseys, crossbreds by Holstein sires, crossbreds by Guernsey sires and all breed groups combined, respectively. (AH g2-23)

(e) Effect of crossbreeding on production. Production records of purebred Red Danes, Milking Shorthorns and Red Polls; and two- and three-breed crosses from the Indiana project were analyzed for the effects of breed of dam, breed of sire, dam x sire interaction, sire in breed, year of calving season of calving, lactation number and age on milk production and persistency of milk yield. The mean milk yield of the two- and three-breed crosses was 880 and 1,550 lb. above the parental mean. The two-breed crosses were similar to the parental mean in persistency of milk yield whereas the three-breed crosses averaged 9% higher. The data indicate an increase of 10.8% in milk and 15.5% in fat production due to crossbreeding. Although the production of the crossbred groups was above the parental mean, none of the groups exceeded the pure Red Danes in total milk yield. The least squares analysis showed that within the purebreds, breed of sire, dams, breed of dam and the interaction effects were consistently significant. Whether the animal was a two- or three-breed cross influenced its performance, but the type of three-breed combination did not greatly influence production.

In the Beltsville experiment, preliminary data on two-bred crosses among Ayrshires, Brown Swiss and Holsteins indicate some increase above the "expected" (contemporary parental mean) in production traits. The Ayrshire x Holstein crosses in first lactation exceeded the parental average by 15 and 14% in milk and fat yield, respectively. The Brown Swiss x Holsteins were +17 and 16%, respectively, while the Ayrshire x Brown Swiss averaged only 10% above the expected in milk and fat. All three groups of crossbreds averaged lower in percent fat than the parental mean (range -.05 to -.11%), but all crosses averaged 10.5% above the expected in persistency of milk yield and 4.8% in gross efficiency (lbs. FCM/therm of estimated net energy consumed). The crossbreds have also averaged 3% above the expected in rate of milk flow (lbs/min). Although the performance of the crosses indicates some heterosis for production traits, none of the crossbred groups have exceeded the purebred Holsteins in rate of milking or total milk production. All crossbred groups have exceeded the Holsteins in fat percent. The Ayrshire x Holsteins have exceeded the Holsteins by 2% in fat production and



the Swiss x Holstein crosses have shown greater persistency than the purebred Holsteins. (AH g2-23)

6. Genetic methods for developing adaptability. These investigations are to evaluate the effectiveness of certain genetic methods for improving dairy cattle adaptability to hot conditions through: (a) introduction of adaptability characteristics; (b) selecting within existing breeds for further adaptability; and (c) hybridization of existing breeds by continuous crossing or developing new strains from a crossbred foundation. This work is in cooperation with the Georgia, Louisiana, and Texas Agricultural Experiment Stations. All of the studies are contributing projects to the Southern Regional Dairy Cattle Breeding Project S-49. Most of the semen services are supplied through the cooperation of the National Association of Artificial Breeders. The PL-480 cooperative project in Colombia also contributes to this work.

(a) Introduction of adaptability by crossing Jerseys with Brahma cattle. Preliminary analysis of the Brahma-Jersey crossbreeding study at Texas A & M show the crossbreds have shorter lactations and average less in milk yield than contemporary paternal half-sib purebred Jerseys. The 1/2, 3/4, 7/8 and 15/16 Jersey crosses averaged 51, 24, 7 and 7% lower in milk production than the purebreds. The crossbreds also calved one month later (29 mo.) and required more services per conception (2.3) than the purebreds (1.8). One-third of the crossbreds have exceeded the average of the Jerseys in milk yield and this groups is now being mated to crossbred bulls to further determine the possibility of development of a desirable strain of cattle from combinations of these two breeds.

(b) Selection within existing breeds. Estimates for heritability of milk yield, using the intra-sire regression method on 510 daughter-dam pairs representing 36 Holstein sires in the Louisiana State University herd for the period 1927-61, were found to be .31, .38 and .40 for first lactation, second lactation and the average of all lactations, respectively. On a single record basis, the heritability for the average of all lactations was .23. The values for milk fat yield were .27, .39 and .28, respectively. Estimates obtained by the paternal half-sib correlation method on 2,243 lactations of daughters of 41 sires were somewhat inflated due to environmental trends in the herd. Repeatability estimates derived by intra-class correlations were .43 for milk yield and .40 for milk fat yield. These values represent estimates without any correction for year-season of calving. The estimates of heritability and repeatability obtained are of comparable magnitude to those for the same traits in cooler climates, thus indicating that expected changes from selection in warm climates is comparable to that of temperate climates. Also, these estimates support the contention that a fairly accurate estimate of the breeding value of a cow can be safely based on the first and/or second lactation yield. (AH g4-2)

(c) Hybridization of existing breeds by continuous crossing or developing new strains from a crossbred foundation. Lactation records made during the period 1957-62 in the herd at Reidsville, Georgia,



were analyzed to evaluate the performance of crossbreds in comparison to purebreds. The foundation females for the crossbreds were Jerseys in all cases. All the crossbred groups (1/2 Swiss-Jersey, 1/2 Holstein-Jersey, 1/2 Swiss-1/4 Holstein-1/4 Jersey and 1/2 Holstein-1/4 Swiss-1/4 Jersey) exceeded the expected (mean of parental breeds) in FCM by 11, 7, 11 and 21%, respectively. The average deviation for the 1/2 Swiss-Jersey group was 525 lb. FCM above the Jerseys and 885 lb. above the Swiss. The 1/2 Swiss-1/4 Holstein-1/4 Jersey crosses averaged 1,224, 787 and 150 lb. above the purebred Jerseys, Swiss and Holsteins, respectively. The 1/2 Holstein-Jerseys exceeded the Jerseys level by 1,048 lb. FCM, but were below the Holsteins by an average of 165 lb. FCM. The 1/2 Holstein-1/4 Swiss-1/4 Jersey group exceeded all other crossbred groups and averaged 2,113, 2,202 and 665 lb. FCM above the purebred Jerseys, Brown Swiss and Holsteins, respectively. The crossbred groups were lower in fat test than Jerseys but higher than the other purebreds. The increase in FCM yields due to crossbreeding (7-21%) are somewhat higher than reported in other studies and the first indication of crossbreds exceeding the Holsteins in total milk and fat yield. These preliminary results suggest that crossbreds are more adaptable to the conditions of Southeast Georgia than purebreds.

At the Iberia Livestock Experiment Station, Jeanerette, Louisiana, preliminary summaries on twenty 1/2 Brown Swiss and thirty-five 1/2 Holstein crosses in relation to their purebred Holstein herdmates showed the Brown Swiss crosses were far below the purebreds in milk and fat yield while the Holstein crosses were slightly below the purebreds in milk, but above in fat yield. Both crossbred groups averaged approximately 0.5% higher in butterfat and solids-not-fat test than the Holsteins. The crossbreds were also higher in persistency of milk yield. Breeding efficiency (services per conception) was high in all three groups, 1.37 for pure Holsteins and the Brown Swiss crosses, and 1.71 for the Holstein crosses. These differences were not significant. Thus far, neither of the crossbred groups has shown any advantage over the purebreds in livability (number reached calving age/number born). The average percent of those born that reached calving age was 88, 80 and 78% for Holsteins, Holstein crosses and Swiss crosses, respectively. In general, the first and second generation purebred Holsteins have exceeded the crosses in growth rate at 6, 12, 18 and 24 months of age. The Holsteins have been significantly heavier in body weight at all ages and have also tended to exceed the crossbreds in body length, and in height at withers. These preliminary results indicate that crossbreds are no more adaptable to Louisiana conditions than Holsteins sired by highly selected sires. (AH g4-2)

(d) Dairy merit of Consteno con Cuernos cattle in Colombia. A deterrent to the usefulness of the Criollo breed, Consteno con Cuernos (CCC), as a dairy animal has been failure to obtain milk let-down without the presence of the calf. At Cerete, an attempt was made to milk 142 first-fourth lactation cows without the calf present. Those milked without the calf produced less total milk yield and had shorter lactations than contemporaries milked with calf at foot. The first lactation animals did somewhat better,



however, than the older cows. Extremely high calf losses under the system of bucket feeding in open corrals were also experienced. Calf losses can be reduced by training personnel but unless some system of management can be devised for obtaining milk let-down without the calf, this may seriously impede the use of the CCC breed in a dairy operation. The 127 CCC heifers that freshened during the year averaged 2 years, 11 months of age. Indications are this can probably be reduced to at least 2 1/2 years since 96 of the animals calved at 31 months or younger. (S5-AH-1)

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Dairy Cattle - Physiology  
Animal Husbandry Research Division, ARS

Problem. Fundamental physiological research is required as a basis for improving lactational and reproductive performance of cattle. Breeding failure is a major reason for the disposal of the physiological action of hormones in controlling reproductive activity, correcting reproductive abnormalities and stimulating lactation. Research on physiological processes related to growth and development, nutritional requirements and to heat tolerance of dairy cattle is required.

USDA PROGRAM

This is a continuing program, almost entirely on basic research, conducted by physiologists and biochemists. The program is designed to elucidate the reproductive and lactational physiology of cattle utilizing physiological and biochemical techniques and to determine physiological mechanisms related to heat tolerance. The work is in progress at Beltsville, Maryland, and co-operatively at the Wisconsin, New York, Massachusetts, Texas, Louisiana, and Georgia Agricultural Experiment Stations. It is coordinated with the NE-41, W-49, and S-49 regional projects.

The Federal scientific effort devoted to the research in this area totals 7.7 professional man-years. Of this number, 2.9 are devoted to the physiology of reproduction, 2.0 to the physiology of milk secretion, 1.0 to the physiology of growth and development, 1.5 to environmental physiology, and 0.3 to program leadership.

A grant with the Veterinary School of the University of Sao Paulo, Brazil, provides for research on the anatomical and physiological characteristics affecting heat production and heat loss of Zebu, European and Zebu-European crossbred cattle and the nature and method of controlling the inheritance. Its duration is for five years, 1961-1966, and involves PL-480 funds with a \$63,293 equivalent in Brazilian Cruzeiros. (Pertains to Area 5 also)

A grant with the Indian Veterinary Research Institute, Izatnagar, UP, India, supports studies on the physiology and genetics of characteristics influencing the adaptability of cattle and buffalo for dairy production. The duration of this work is five years, 1961-1965; it involves PL-480 funds with a \$195,624 equivalent in Indian rupees. (Pertains to Area 5 also)

A grant was initiated this year with the Veterinary Institute, Beit Dagan, Israel, in the Department of Reproduction, for work on a project entitled "Comparative Studies of Repeat Breeders and Normal Cows and Heifers." It is for a five-year period and involves PL-480 funds to the extent of \$124,600 equivalent in Israeli pounds.

A grant was initiated with PL-480 funds with the Department of Applied Pharmacology, The Hebrew University, Rehovoth, Israel, on the mechanism of lactation and its augmentation by hypothalamic stimulation. It is supported for five years and is for \$126,767 equivalent in Israeli pounds.

## REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

### A. Physiology of Reproduction

A large portion of this work is reported in Area 1, Animal Biology, because it has application to classes of livestock other than dairy.

#### 1. The corpus luteum

(a) The role of progesterone in maintenance of the corpus luteum of the estrual cycle. The role of progesterone in luteal maintenance during the ovine estrual cycle was studied by comparing spontaneously formed corpora lutea with corpora induced experimentally by injection of gonadotropin. Corpora lutea formed spontaneously after removal of natural corpora functioned for the length of a normal cycle from the time they were formed. Experimental corpora lutea induced on days 5, 7, or 9 of the estrual cycle, while natural ones were present, became functional, but did not modify the cycle length. When progesterone was given from days 4 to 7 and natural corpora lutea were removed on day 5, corpora induced on day 7 also became functional and did not modify the cycle length.

In other experiments progesterone was given from days 11 through 22 and induced corpora were formed on day 21. These induced glands usually failed to show any evidence of function or of being maintained. Estrogen treatment, electrical stimulation (per rectum), or both, given while the induced corpora lutea were being formed, failed to establish maintenance of these glands.

(b) Progesterone content of corpora lutea and ovarian effluent blood. Progesterone levels in corpora lutea were studied at 4, 6, 8, 10, 12 and 14 days after estrus in 38 normal ewes. The average weight and total progesterone content of corpora lutea were affected by stage of the cycle ( $P < .01$ ).

Relationships also were determined between hormone content of corpora lutea and ovarian effluent blood plasma progesterone at 6, 10, and 14 days of the cycle in normal and superovulated ewes. The total luteal weights of superovulated ewes decreased significantly from day 6 to 14 after ovulation. Blood flow rate in these ewes at the same stage of the cycle was correlated with total luteal weight ( $0.56$ ,  $P < .01$ ). The secretory rate (amount of progesterone in effluent plasma/unit time) was significantly correlated with the luteal progesterone content ( $P < .05$ ).

The data support the hypothesis that luteal progesterone content is indicative of the level of function of this gland in terms of the amount of hormone released into the blood.



(c) Effects of experimental endocrine treatment of animals upon the activity of their corpora lutea. Attempts have been made to modify corpus luteum size and activity with several hormonal substances which may be involved in the function and maintenance of this gland. Progesterone levels in the gland and the amounts of these steroids produced in vitro during incubation of luteal tissue have been used as measures of luteal activity.

(1) Oxytocin. Control CL (day 14) and CL obtained on day 14 of a cycle after oxytocin treatment on days 12 and 13 showed little differences in weight, initial progesterone content or progesterone content after in vitro incubation with ATP, DPN, and pregnenolone.

In another series of experiments the progesterone level was measured on the 11th day of the cycle in Holstein heifers treated daily with oxytocin or with daily oxytocin injections plus daily injections of the anterior pituitary preparation being tested for its ability to overcome the inhibitory effects of oxytocin on the corpus luteum. Oxytocin treatment reduced the mean total progesterone level from 262  $\mu$ g in control CL to 30  $\mu$ g.

Prolactin, somatotrophin and equine LH were not luteotrophic in the bovine, since they failed to overcome oxytocin induced inhibition of luteal development and progesterone production. On the other hand, HCG and a crude bovine anterior pituitary extract were strongly luteotrophic and resulted in an increase in mean total progesterone.

Histologically, the new corpora lutea formed during oxytocin treatment closely resembled normal corpora lutea collected from untreated cattle at 2-3 days post-estrus. The progesterone content of these new corpora lutea from oxytocin treated heifers was also quite similar to that found in normal corpora lutea of a similar age. These observations suggest that the inhibitory effect of oxytocin on luteal development and progesterone production does not become effective until the third day after estrus.

(2) Luteinizing hormone (LH). Growth of normal corpora lutea was most rapid during the 8 days following ovulation. Day 6, 8, and 10 CL removed from rabbits treated with a single intravenous injection of LH 48 hours previously were significantly smaller than CL of a comparable stage of growth. Day 6 LH treated CL were retarded in growth, day 8 CL exhibited luteostasis (no change in weight) and day 10 CL had regressed. LH injected into 9 day pseudopregnant rabbits caused regression of natural CL but, at the same time, induced the formation of new CL. These induced CL continued to grow and did not regress at day 16-17 of that cycle, thus indicating that the natural mechanism by which the life span of natural corpora lutea is terminated had no effect on the ability of the induced corpora lutea to reach maturity. (AH h5-6, cooperative with the University of Wisconsin).

2. The anterior pituitary-ovarian interrelationship in experimental endocrine states. The ripening of an ovarian follicle and the process of ovulation is dependent upon the secretions of the anterior pituitary gland. The relationship of the resultant corpus luteum to the anterior pituitary was studied by determining the relative content of follicular stimulating hormone (FSH) in the pituitary after progesterone treatment. Progesterone treatment from day 7 of the cycle to day 41 depressed both the size of the follicles and the total follicular fluid weight. FSH and luteinizing hormones (LH) concentrations were not affected by progesterone administration. The injection of oxytocin from the day of heat to day 6 of the cycle produced a smaller CL and a reduced progesterone content but had no effect on hypophyseal FSH and LH.

The pituitary-ovarian relationship was also studied during the perinatal period. CL from a group of cows slaughtered at 20 days pre-partum showed 26 µg progesterone per gram of CL tissue. CL from the day of calving had no detectable progesterone. Preliminary analysis of pituitary assay data showed that the pre-partum and day of calving groups had comparatively more FSH and less LH than did the 21 day post-partum group. (AH h5-6, cooperative with the University of Wisconsin).

3. Hypothalamus-pituitary-corpus luteum interrelationships. Experiments utilizing renal autografts of anterior pituitary glands were designed to determine how the hypothalamus and higher nerve centers influence pituitary gonadotrophin secretion and ovarian activities. These rats bearing renal autografts of anterior pituitary glands provided an in vivo system for studying the effects of various hypothalamic substances on the isolated pituitary. Corpora lutea were maintained for prolonged periods of time in the autografted rats, as had been previously reported, while corpora from hypophysectomized rats were less well maintained.

Homogenates of bovine hypothalamic tissue, oxytocin and vasopressin all caused nearly complete gross and histological regression of corpora lutea. The results suggest that the hypothalamic homogenate and the oxytocin and vasopressin preparations used all contained substances capable of stimulating the transplanted pituitary gland to produce a luteolytic hormone. In another experiment conducted the same way, the uteri were traumatized to determine whether decidual cell reactions would be induced. Deciduomata formed in 68 percent of the untreated rats bearing autografts, proving that the corpora lutea in these animals continued to secrete progesterone.

The findings that injections of bovine hypothalamic extracts and vasopressin cause regression of the corpus luteum in the rat under several experimental conditions are of considerable theoretical interest, and may eventually contribute to an understanding of how the hypothalamus influences the release of anterior pituitary hormones. (AH h5-3 Cooperative with Cornell University).



4. Experimental alteration of the estrous cycle in cattle. This work is part of a study to determine the factors which control ovulation and regulate the estrous cycle in cattle. Two commercial orally active progestational compounds, Provera and Lutinyl (CAP), were fed to Holstein heifers to regulate the estrous cycle. Provera was fed to 19 Holstein heifers for 14 days. All heifers came in estrus 2-6 days after the hormone was withdrawn and 9 (49%) of them conceived when bred at this time. This treatment appears to be less desirable than those previously reported, since the heifers came in estrus over a slightly longer period of time, and the fertility was slightly lower.

Major interest during the year has centered around a highly active chlorinated progestin, Lutinyl or CAP. The following conclusions are drawn from these experiments: (1) This compound effectively inhibits estrus in Holstein heifers and cows when fed at rates as low as 12 mg. per day. (2) Fairly good synchronization of estrus is obtained within a 3-day period after the hormone is withdrawn. (3) The conception rates of the cattle bred at the artificially controlled estrus vary from 50 to 60 percent. (4) Subsequent cycle lengths are normal and indications are that normal conception rates (60-70%) are obtained in heifers bred at the first subsequent estrus. This compound appears to be potentially useful for estrous cycle synchronization in dairy cattle and its potency may make its use economically feasible.

A review of all of the cycle synchronization trials conducted to date suggests that animals are slower to come in estrus and less well synchronized when the hormone is fed mixed with the entire daily grain ration than when it is fed individually in small amounts of concentrate. The finding that fairly good synchronization and fertility can be obtained with a total of only 216 mg. of hormone per animal suggests that cycle synchronization may become practical from an economical standpoint.

Administration of oxytocin on days 3 through 6 of the estrous cycle was effective in shortening the length of the cycle. Simultaneous administration of epinephrine (8 mg daily) or atropine (50 mg daily) prevented this action. Epinephrine alone on these days has no effect on cycle length; the effect of atropine given alone is currently under study. (AH h5-3, Cooperative with Cornell University and AH h5-4 Cooperative with the University of Massachusetts)

5. Sperm transport through the tubo-uterine junction. Studies were initiated to determine spermatozoan transport through the tubo-uterine junction of rats and cattle. Immotile rat, rabbit, bull and human spermatozoa when introduced into the tubo-uterine junction did not appear to select against the passage of foreign sperm, nor was motility essential for transport.

Sperm transport has also been studied in the immature bovine. Motile bull sperm were found in the oviducts of young calves (29-128 days of age) within 13 minutes (the earliest time studied) after vaginal deposition. No difference in transport time was found between hormonally treated (various combinations of PMS, HCG, progesterone and estrogen) and untreated animals.

Leucocyte invasion of the vagina was noted in these calves. In a further study of this phenomenon, maximum influx was found to occur between 4 and 8 hours after vaginal insemination. Bacteria-free semen was capable of initiating this response, while introduction of the diluent or sterile physiological saline was ineffective.

Secretory activity of the oviduct is being studied by means of a collecting device attached to the flank of the animal and connected to the oviduct by a polyethylene catheter. In sheep a maximum secretion rate of 0.085 ml/hr. occurs at estrus, with a minimal rate of 0.010 ml/hr. at mid-cycle. Collected secretions are being analyzed for quantitative changes associated with the cycle. Similar procedures are being applied to cattle, and it is the further hope that the influence of these secretions on sperm and ovum viability and metabolism can be determined. (AH h5-4, Cooperative with the University of Massachusetts).

## B. Physiology of Milk Secretion

1. The histamine concentration in the milk, blood and urine of dairy cattle. The histamine levels in milk, blood and urine have been determined as part of studies on the role of histamine in secretory and other processes in dairy cattle. Blood histamine values in 19 growing Holstein heifers, from birth to 45 weeks, averaged 0.3 µg/ml. Eight bulls showed similar blood values of 0.3 µg/ml. Blood levels during this rapid growth period showed little change, values being slightly lower during the earlier weeks. Blood histamine concentration of 5 animals sampled at 6 hour intervals for 3 days indicated little diurnal variation. Such repetitive sampling was, however, associated with a progressive increase in histamine level. Twenty-five milk samples from 15 cows were analyzed for histamine, the mean concentration being 0.5 µg/ml. When the fluorimetric histamine procedure was applied to urine, 4 samples from heifers fed alfalfa-grass hay showed a mean concentration 1.1 µg/ml while 4 samples from animals fed alfalfa-grass silage were 19.5 µg/ml. These concentrations averaged about 20 to 30 times those found in blood. Computed daily urinary excretion was 2-12 mg and 48-356 mg respectively for the hay and silage fed animals. (AH h5-1)

2. The mechanism of lactation and its augmentation by hypothalamic stimulation. The interrelationship of the hypothalamus and the anterior pituitary gland in stimulation and maintenance of lactation was studied by depressing the activity of the hypothalamus with tranquilizers and other drugs. Reserpine, nine phenothiazine derivatives and five tranquilizers and Librium were studied for their mammatropic and lactogenic effects. In adequate doses, reserpine was found to induce lactation along with morphological and histological changes in the ovaries and uteri similar to those encountered in lactating rats. In pseudopregnant rabbits reserpine caused continued lactation.

In an initial screening of the 16 drugs perphenazine was found to be most effective and accordingly, further studies will be concentrated upon this



compound. Rats and rabbits treated with perphenazine for 1 week reacted with copious milk secretion. Preliminary results indicate that this lactation is due to a prolactin-releasing factor of the hypothalamus, since extracts of such hypothalami produce lactation in rats. (A10 - AH-3 - Israel)

### C. Physiology of Growth and Development

The effects of nutrition during early growth and development on reproductive performance. The studies of reproductive performance of cattle fed Low, Medium and High levels of nutrients during early life have continued. Of the 34 cows fed Low, Medium, and High levels of nutrients during early life, only 9, 4, and 4, respectively, remain in the herd. The numbers of cows that have become sterile are: Low, 0; Medium, 4; and High, 8. No difference in milk yield in any of the first four lactation periods has been observed between any two of the early-life treatment groups. However, the output of milk per unit of metabolic size has been somewhat lower for the High cows than for the other two groups, in all lactations.

The most important observations made to date are as follows: (1) Heifers smaller than normal at the time of first calving reach normal size by the time of the third calving when fed liberally after the first calving. (2) Cows, while calving, have produced at least as much milk through the sixth lactation as cows which were fed liberally throughout. (3) When heifers are not bred earlier than 18 mos. of age, high-level, early-life feeding is impractical. (4) Based upon the production response through the sixth-lactation period, heifers can be raised quite satisfactorily on as little as 150 lbs. of milk (with limited amounts of dry concentrates) and an all-roughage ration after 6 mos. of age. (5) Low levels of nutrients early in life retard sexual maturity. (6) The level of feeding early in life did not affect the rate of conception for the first pregnancy. (AH h5-3 Cooperative with Cornell University).

### D. Environmental Physiology

These studies deal with the determination of the anatomical and physiological characteristics affecting heat production and heat loss of dairy cattle under hot conditions and the nature of the inheritance of these characteristics. Work is in progress at Beltsville and in cooperation with the Georgia and Louisiana Agricultural Experiment Stations. These studies contribute to the Southern Regional Dairy Cattle Breeding Project, S-49. Cooperative projects sponsored under PL-480 grants in Brazil and India also support this work.

1. Methods of determining thyroid functions of animals under heat stress. Studies were conducted at Louisiana State University to evaluate the accuracy and repeatability of five methods for determining thyroid functions. The methods compared were thyroid secretion rate (substitution), thyroid utilization rate, estimated thyroxine secretion rate, serum protein bound iodine (PBI) and metabolic heat production. All the methods



were evaluated under cool conditions (60-70°F.) and at 90°F. The results indicate the most desirable method for determining thyroid function under heat stress was thyroxine secretion rate (substitution) method. Thyroxine utilization rate was found to have a non-significant positive correlation with both the thyroxine secretion rate (substitution) and metabolic heat production methods and a negative correlation with PBI. Estimated thyroxine secretion rate and metabolic heat production were found to have a significant positive correlation of .79 under both temperature conditions and, where accurate PBI data were available, could be used to determine thyroid function. Metabolic heat production may be used under uniform cool conditions to determine thyroid function, but was found to be unreliable when animals were under heat stress. PBI did not appear to be an accurate measure of thyroid function under either the cool or hot conditions. (AH g4-1)

2. Effect of photoperiod, temperature and humidity on the semen production of dairy bulls. A study was made at Louisiana State University to determine the effect of daily photoperiod and temperature on fertility of dairy bulls from May-October. Three groups were used; one kept in a barn under prevailing daylight and temperature conditions; the second in the same barn with the daylight increased gradually at 7-day intervals from 14 to 16 hours; and a third group was kept in a climatic control chamber at 64°F. with the length of the daylight period increasing from 14 to 16 hours. The percentage of shippable ejaculates was used to evaluate the treatments. The group under the regular barn conditions averaged only 18.3% shippable ejaculates while the increased photoperiod group averaged 32.6%, and those kept at 64°F. averaged 75.5%. These tests indicate that both length of photoperiod and temperature are factors in the production of good quality semen by dairy bulls during the summer months. (AH g4-1)

3. Some physiological responses of Indian cattle and buffaloes to heat stress. Studies of the responses of various breeds of cattle and buffalo are being conducted at the Indian Veterinary Institute, Izatnagar, India, under a PL-480 grant. Groups of yearling heifers represented the Harijana, Kankrej and Tharparkar breeds of cattle and Murrah buffalo have been subjected to cool conditions (70°F.) and 6-hour periods of hot-dry (120°F. dry bulb with 15% relative humidity) and hot-humid (105°F. dry bulb and 70% humidity) conditions in a psychrometric chamber. Exposure to both the hot-dry and hot-humid conditions resulted in significant changes over the cool conditions but the responses of the three breeds of cattle were very similar. The degree of response in the buffalo differed, however, from the cattle in several respects.

In the cattle, both skin temperature and coat radiations showed significant rises under the hot conditions with the responses under the dry and humid conditions being similar. Under the high temperatures there were declines in hematocrit and hemoglobin with the greatest decline under the humid conditions. There was also a significant decrease in erythrocyte counts under the hot conditions. Under the hot-humid conditions significant drops in calcium and inorganic phosphorus were observed whereas under the hot-dry



atmosphere the calcium and phosphorus levels increased. A rise in blood glucose occurred at the high temperatures with somewhat higher levels under the humid conditions. Blood and plasma volume showed significant increases at the high temperature.

From the studies thus far, the most significant factors in behavior of these Zebu breeds as compared to results reported for European breeds have been an increased pulse rate with exposure to hot conditions and higher rates of sweating, but more data will be needed for verification. Data on skin temperature, rectal temperature and blood constituents are within the limits reported for European breeds.

The rectal temperature of the Murrah buffalo heifers was somewhat lower than for cattle under the cool conditions but under the test conditions the buffalo had higher rectal temperatures. There was a marked difference in the respiration rates of buffaloes and cattle upon exposure to the hot conditions with the buffaloes having the higher rates. Buffaloes showed a lower pulse rate under cool conditions and the change in rate with exposure to hot conditions was of a much lower magnitude than in cattle. Respiratory minute volume of the buffalo was appreciably lower under cool conditions but significantly higher than for cattle under the hot-humid and hot-dry conditions. The differences in response of the two species in respiratory minute volume seems related to differences in respiratory rate. The skin temperature of the Murrah heifers increased about the same as that for cattle under the hot conditions. The changes in hematocrit, hemoglobin and blood glucose followed similar patterns to that of the cattle with the buffalo values being somewhat lower. (A7 AH-1)

4. Adaptability studies in Brazil. The adaptability of European breeds (Brown Swiss, Holsteins, and Jerseys); Zebu breeds (Nellore and Kankrej); and crosses of European and Zebu, to conditions in Southern Brazil is being studied at the Instituto de Zootecnia at Pirassununga, Brazil.

(a) Reproduction of Jersey cattle. Studies on the reproductive performance of Jerseys, two or more generations away from importation, indicate that breeding efficiency compares favorably with that reported for temperate areas. The average services for conception, over all gestations, was 1.54. The average age of first conception was 19 months, and the mean age at first calving was 29 months, but the interval from calving to conception in second and later gestations was longer than desired (113 days) due to silent heats or no ovulation. The delayed breeding resulted in a calving interval of slightly over 14 months. The average gestation period ( $274 \pm 1.05$  days) was somewhat less than reported for Jerseys in temperate climates. The workers are of the opinion that conditions of climate in Brazil are not severe enough to interfere with sexual maturity, but a combination of climate and nutrition are probable factors in delaying conception during lactation.

(b) Hair coat characteristics. Development of a very short hair coat seems to be one of the adjustments European breeds make when introduced into tropical or sub-tropical areas. A study of the hair coat depth of imported Brown Swiss versus those reared in Sao Paulo state for two generations indicated the imported animals had shorter hair coats after one year but not as short as those born in Brazil. The mean hair coat depth for the imported stocks was 0.55 inches during winter and 0.24 inches in summer, whereas the means for the local reared animals were 0.21 and 0.11 inches. Brazil reared Holsteins and Jerseys also had very short coats but not as short as for the pure Zebus which were too short to measure. Crosses of 1/2, 3/4 and 7/8ths Holstein-Zebu breeding by imported Holstein bulls had hair coats similar to that of second and later generation Brazil Holsteins. Results to date indicate both genetic and environmental influences are involved in hair coat depth. Further, it appears that the time lapse for imported cattle to develop a short hair coat has a direct relation to their survival and performance in Brazil. (S3-AH-7)



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**DAIRY CATTLE - NUTRITION AND MANAGEMENT**  
**Animal Husbandry Research Division, ARS**

Problem. Information on the nutritional processes and requirements of dairy cattle is needed to obtain a more precise evaluation of feeds and rations as a basis for improving feeding practices on farms. Shifts in sources of nutrients fed to dairy cattle require studies on the optimum combination and specific supplements needed in order to provide for the most profitable production. Also, dairymen need to reduce costs including man-hours of labor and develop better management in the use of improved types of dairy equipment and feeding, bedding, and milk handling systems.

**USDA PROGRAM**

The current program is conducted by biochemists, nutritionists and dairy husbandmen. At Beltsville, studies are in progress on the factors which contribute to the heat production and maintenance requirements of grazing animals; the relationship between net energy, metabolizable energy, and total digestible nutrient values in dry roughages, silages, green roughages, and concentrates; and the relationship between digestibility and the chemical composition and solubility of various feed constituents. Calorimetric techniques are being applied to studies on the effects of dietary and physiological factors on energy metabolism and requirements of cattle. A cooperative project at Tifton, Georgia, has been recently initiated on the residues in milk resulting from the ingestion of pesticides and herbicides associated with the treatment of animals and crops.

At Beltsville, Maryland, research is being conducted on the effects of crop maturity, moisture content, preservatives, including methods of handling and conditions of storage, on the chemical quality, palatability and feeding value of silages. In conjunction with this effort, biochemical studies are being made to determine the effect of the composition of forage at the time of ensiling and of varying imposed conditions on the composition of the resulting silage. Related to the Beltsville studies is cooperative work at Lewisburg, Tennessee; Willard, North Carolina; and Puyallup, Washington. The objective of the work at the Tennessee station is to determine the effectiveness of various practical ensiling procedures by varying such factors as moisture, preservatives, type of silo, etc. At North Carolina, comparisons are being made of upright and bunker type silos. At Washington, the scientists are studying comparisons of bunker and tower silos. Pasture studies at Beltsville, Maryland, involve the effect of varying stocking rates on nutrient yields per acre and on production per animal. In cooperation with the Washington State Experiment Station at Puyallup, Washington, work is being carried out to determine dry matter consumption and digestibility of nutrients from pasture species.



A cooperative project at Logan, Utah, has been undertaken to measure the variations in efficiency of forage utilization by dairy heifers and to determine the factors which account for these variations.

At Beltsville, Maryland, a continuing study is underway to obtain information on the extent of the variation in amount of dry matter and total digestible nutrients the dry, non-pregnant, mature cow requires to maintain body weight under practical conditions and to study and evaluate various factors that may influence the maintenance requirements.

The work at Beltsville, Maryland, also consists of studies on wilted silage as a forage for growing dairy heifers, the vitamin and mineral requirements of calves and deficiency symptoms using a synthetic type of diet with particular emphasis on vitamin A and magnesium deficiency. At Willard, North Carolina, the research involves pasture utilization by young, growing cattle.

Scientists at Beltsville are engaged in studies on the environmental conditions and the mechanisms of infection involved in bovine mastitis. They are also making a comparison of hand vs. machine milking of dairy cattle. In cooperation with Agricultural Engineering, Entomology, and Eastern Utilization, research is in progress on electrically-controlled and operated equipment for reduction of labor in dairy cattle management; on the evaluation and development of physical methods for control of flies and other dairy cattle pests; and on the relationship between management practices and milk quality including flavors.

Cooperative work with Agricultural Engineering and with the Georgia Coastal Plain Experiment Station is being conducted on the influence of management practices and other environmental factors on the adaptability of cattle to the Southeastern United States.

The Federal scientific effort devoted to research in this area totals 25.4 professional man-years. Of these 7.0 are in digestion and metabolism, 8.1 in forages, 3.1 in nutritional requirements, 3.6 in calf feeding, 2.7 in management practices, and 0.9 in program leadership.

A grant with the Government Agricultural College and Research Institute, Ludhiana which is affiliated with Punjab University, Chandigarh, Punjab, India, provides for research on factors affecting the utilization of low-grade roughages and production of volatile fatty acids in the rumen of cattle. Its duration is for five years, 1962-67, and involves PL-480 funds with a grant of \$86,598 equivalent in rupees. (Pertains to Area 1 also).

## REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Digestion and Metabolism

The question concerning the most accurate method of evaluating and expressing the energy values of feeds has not been settled. Some use total digestible nutrients while others have suggested that metabolizable energy or net energy are more suitable for evaluating feeds. There is also the question of supplementary effects between feeds (i.e. forages and concentrates) as well as the question of efficiency of utilization of energy for the various productive uses such as fattening, growth, or lactation. The energy laboratory was established to answer these questions and research is in progress on each of the above problems. The influence of dietary factors on the utilization of energy for lactation is being studied and a considerable amount of data has been collected during the past year.

1. Development of computer programs for energy balance trials. The routine calculations associated with energy balance trials are extensive and of a repetitive nature. The time required to perform these computations forms an important portion of the total labor involved in energy metabolism experiments, so computer programs were developed to automatically decode, calculate, and summarize data. These programs should prove useful not only to increase the speed, reliability, and ease of subsequent summarization and statistical treatment of the data collected in the USDA Energy Metabolism Laboratory, but should also be helpful to other investigations in which digestion and balance trials are conducted. A considerable amount of time has been spent in developing the computer programs, since over 400 individual equations were developed and programmed to decode automatically recorded data, calculate respiratory exchange, combine feed, orts, feces, milk, and related data and summarize the carbon, nitrogen, and energy balance experiments. Other research programs in which only digestion trials or nitrogen balance experiments are conducted can use a portion of one of the programs for computations and summaries.

Three programs are now being used. The first, program "A", is used to decode, calculate, and summarize the gas volumes and respiration chamber conditions. During a respiration trial, 240 cards per animal per day are punched automatically. These are processed at the rate of 175 cards per minute, or 1.33 minutes per animal per day. The "B" program is then used to combine the gas composition and related information with the corrected gas volume, and to compute the oxygen consumption, carbon dioxide, and methane production, gaseous carbon excreted, total heat production and other information. Since July 1962 these two programs have been used regularly, with the results being obtained on the same day the respiration trial ended.



The third program, "C", was prepared to combine all the data collected during carbon-nitrogen-energy balance studies and to compute digestion coefficients, utilization of carbon, nitrogen, and energy, maintenance requirements of the animals, distribution of losses of carbon, nitrogen, and energy, the metabolizable energy of the rations, energy balance, net energy, and various other information. If respiration data is not available this program may be used to summarize digestion trials and nitrogen balance experiments.

With the completion of these programs, it is now possible to compute and summarize energy balance trials in only 30 minutes, as compared to the three months required formerly to perform these computations manually. (AH h2-8)

2. Fasting metabolism of dry non-pregnant cows. The fasting metabolism of the adult ruminant has been the subject of investigations by physiologists and nutrition workers for over a century. The fasting (basal) metabolism has been used extensively for computing the effects of muscular work, lactation, gestation, maintenance, temperature regulation, growth, and specific nutrients on heat increment. Therefore, the conditions which affect this value must be understood.

The fasting metabolism of six dry, non-pregnant Holstein cows was measured in 84 24-hour respiration trials. The effects of previous plane of nutrition, type of ration, season (periods), and individuality (cows) were studied, with the basic design being a 3 x 3 Latin square and with the rations being early-cut alfalfa, late-cut alfalfa, and late-cut orchardgrass hay, each being fed as pellets. Each ration was fed at three planes of nutrition, ad libitum, maintenance, and one-half maintenance, and the total fasting heat production was measured 48-72 and 72-96 hours after the last meal.

If the fasting metabolism of the average for the three levels of nutrition is used to express the energy requirement for maintenance, a 1,000 lb. cow ( $98 \text{ Kg}^{0.75}$ ) would require 7.23 therms of net energy. If the fasting metabolism following each of the planes of nutrition fed were used, a cow receiving forage ad libitum would require 7.5 therms per 1,000 lb., one on maintenance rations would require 7.2 therms, and it would be 7.0 therms following one-half maintenance ration. These data, therefore, indicate the previous level of nutrition has an effect on the maintenance value obtained by fasting.

An entirely different requirement is obtained if the "theoretical fasting metabolism" is calculated by extrapolating to zero intake from heat production measurements made for the different rations at various planes of nutrition. Regression equations were computed for each ration, and the theoretical heat production at zero intake was  $57.1 \text{ kcal/W}_{\text{kg}}^{0.75}/24 \text{ hr.}$  for early-cut alfalfa, 56.8 for late-cut alfalfa, and 53.0 for late-cut orchardgrass. The net energy requirements for maintenance of a 1,000 lb. cow would

then be 5.6, 5.6, and 5.2 therms, respectively, for early-cut alfalfa, late-cut alfalfa, and late-cut orchardgrass. The difference between these estimates (actual fasting as compared to extrapolation to zero intake) is due to the difference in the efficiency of utilization of metabolizable energy above and below maintenance. The lower values obtained by rectilinear regression rather than by direct measurement of fasting metabolism reflect the lower efficiency of utilization of metabolizable energy for body gain (lipogenesis). This is primarily because of the "sparing action" of body tissue which is catabolized when less than a maintenance level of feed is consumed. (AH h2-8)

3. Net energy values of selected forages. The net energy values for the all-forage rations fed in the previous experiment have been calculated using different bases for computing heat increment. The highest net energy values were obtained when the fasting metabolism measurements were used as the base, and the lowest values resulted when the heat production of animals being fed at approximately maintenance level was used as the base for calculating heat increment. The efficiency of utilization of metabolizable energy varied from 48% to 71%, depending upon which base was used to compute heat increment.

Regression equations were calculated to obtain the mean heat increment for each ration. This amounted to 0.985 kcal/gram dry matter for early-cut alfalfa, 0.886 for late-cut alfalfa, and 0.804 for late-cut orchardgrass. The mean metabolizable energy values at maintenance were 2.132, 1.833, and 1.788 kcal/gram dry matter, respectively, for the early-cut alfalfa, late-cut alfalfa, and late-cut orchardgrass. The net energy values at maintenance, therefore, are 1.147, 0.947, and 0.984 kcal/gram dry matter, or 52.1 therms/100 lb. early-cut alfalfa, 43.0 therms/100 lb. late-cut alfalfa, and 44.7 therms/100 lb. late-cut orchardgrass hay, expressed on a dry matter basis. The average efficiency of utilization of metabolizable energy ( $\frac{NE}{ME}$ ) using these values is 53.8%, 51.7%, and 55.0%, respectively, for each of the three rations.

Regressions were also calculated to determine the relationship between metabolizable energy intake and retention or loss of energy. Correlation coefficients of 0.975, 0.967, and 0.959 ( $n = 18$  in each case) for early-cut alfalfa, late-cut alfalfa, and late-cut orchardgrass, respectively, were obtained. The average efficiency of utilization of metabolizable energy over the entire range of one-half maintenance to ad libitum was 56.2% for early-cut alfalfa, 58.1% for late-cut alfalfa, and 56.7% for late-cut orchardgrass. These results indicate that there was no significant difference in the efficiency of utilization of metabolizable energy for fat deposition which could be attributed to the type of forage.



4. Metabolizable energy requirements for maintenance. The maintenance requirement of dry, non-pregnant Holstein cows receiving all-forage rations was also calculated from data in the previous experiment. If the rectilinear regression of energy retention to zero is used, the mean maintenance requirements, expressed as therms of metabolizable energy per 500 Kg.<sup>0.75</sup>, varied with rations, being 11.3, 12.4, and 11.0 for early-cut alfalfa, late-cut alfalfa, and late-cut orchardgrass, respectively. For a 1,000 lb. cow, this amounts to 10.5, 11.5, and 10.2 therms of metabolizable energy to prevent loss of body tissue.

The maintenance requirement was also calculated by correcting to zero energy balance by multiplying the energy balance by factors ( $c = 1.61$  for positive balance;  $c = 1.43$  for negative balance) and adding or deducting that amount from the metabolizable energy consumed. The mean values, expressed as Mcal. metabolizable energy/500 Kg.<sup>0.75</sup>, for each plane of nutrition (ad lib, maintenance, one-half maintenance) and ration were as follows: Early-cut alfalfa, 11.4, 10.8, 10.7; late-cut alfalfa, 12.3, 11.5, 11.2; late-cut orchardgrass, 11.0, 10.0, and 10.1. There were no statistically significant differences due to ration, cows, or periods except on half maintenance rations. There was a period effect on one-half maintenance, and this was due to the exceptionally high maintenance requirement of one cow in period I, immediately after she stopped lactating. This same cow also had an apparently high fasting metabolism during the first experimental period. The conclusion drawn from these studies was that the stage of maturity and type of forage did not appreciably affect the utilization of metabolizable energy for maintenance. (AH h2-8)

5. Ad libitum consumption of forages. The ad libitum consumption of the all-forage rations was measured, and the late-cut alfalfa hay pellets were consumed in the largest quantities, followed by early-cut alfalfa hay pellets and late-cut orchardgrass hay pellets. The average dry matter consumption per unit of metabolic body weight (grams dry matter/ $W_{kg}^{0.75}/24$  hr) was 87.2 for early-cut alfalfa, 102.8 for late-cut alfalfa, and 84.3 for late-cut orchardgrass. The gain in body tissue resulting from the ad libitum consumption of the forages, expressed in megacalories/24 hr. was 5.7 for early-cut alfalfa, 5.7 for late-cut alfalfa, and 2.9 for late-cut orchardgrass. Therefore, even though the digestibility, metabolizable energy, and net energy values of the late-cut alfalfa, and late-cut orchardgrass hay were very similar, the late-cut alfalfa hay was markedly more acceptable to the cattle, resulting in much greater consumption of feed and tissue deposition. There was a highly significant difference between cows in their consumption of the rations, and the differences between rations were also highly significant ( $P < 0.01$ ). (AH h2-8)

6. Utilization of energy by lactating dairy cows. Energy balance experiments with lactating cows were initiated in 1962 to study the efficiency of utilization of energy of rations with varying hay:grain ratios. The rations consisted of (1) 100% alfalfa hay wafers, (2) 75% alfalfa + 25% concentrates (corn-soybean oil meal mixture, (3) 50% alfalfa + 50%



concentrates, with the proportions being calculated on an estimated net energy basis. The results of the experiments are not complete, but preliminary summarization of the data indicates that the efficiency of utilization of metabolizable energy for lactation for all forage rations is less than for those containing concentrates if a fixed maintenance requirement (131 kcal M.E./W<sub>kg</sub><sup>0.75</sup>/24 hr.) is assumed.

The maintenance requirements of lactating cows, as determined by rectilinear regression to zero energy balance, are higher than for dry, non-pregnant cows, according to the results obtained to date. The overall mean of 31 observations with lactating cows indicates that the maintenance requirement is approximately 17 megacalories of metabolizable energy per 500 Kg<sup>0.75</sup> as compared to 11-12 mcal M.E./500 Kg<sup>0.75</sup>/24 hr. for dry cows.

The experiments are still in progress and the same animals will be used in further studies with these rations after they complete their lactation periods. (AH h2-8)

#### 7. Development of analytical methods used in energy balance trials.

It is necessary to continually review and study techniques and methods used in order to improve accuracy, to decrease the time required for each determination, and to tie the methods used, if possible, into the computer system by automatic recording.

(a) Gross energy. The routine method of preparing samples for gross energy analysis has been to dry them. Previous experiments on methods of storage and preparation of fecal samples showed that only slight losses of energy occurred during drying, so this procedure was adopted in preference to the use of primers. Urine and milk samples were dried in several different manners, and the results were comparable whether the drying temperatures were 20°C or 65°C. No difficulties in combustion were encountered, even though cellulose blocks or other primers were omitted during the sample preparation.

(b) Carbon. Further advancements have been made in the use of the induction furnace and gasometric analyzer for determining the carbon content of feeds, excreta, and milk. It is now possible, as a result of modification of the equipment and the use of different catalysts, to use this method for wet materials such as urine, milk, and fresh feces and obtain agreement between replicates without drying them, which considerably reduces the time required for the determination. It was necessary to modify the combustion tube and the catalysts before quantitative recovery of carbon from wet materials could be obtained.

It was found that urine and milk samples could not be stored without changing the carbon values, even though they were frozen or refrigerated after being



weighed. The routine procedure adopted was to analyze all urine and milk samples immediately. Since a sample can be analyzed in approximately eight minutes, this did not present a major problem. (AH h2-8)

8. Development of chemical methods for determining the nutritive value of feeds and forage. The nutritive value of forages in particular varies markedly dependent upon stage of maturity, species, method of preservation, and fertilizer practices. Thus it is highly desirable to have rapid chemical methods which will predict the nutritive value of forages. Work in this area has been in progress for about ten years at Beltsville and it now appears that a breakthrough is in the making.

(a) Acid-detergent fiber and lignin. During the past year further analyses were made using the new acid-detergent fiber and lignin procedures. The acid-detergent fiber has compared favorably with the AOAC crude fiber on 72 forages of known digestibility. Correlations were -0.79 for the new method and digestibility and -0.70 for the AOAC method and digestibility. Correlations using the lignin figures have not been made because of large interspecies variation.

A regression equation has been calculated for estimating crude fiber from acid-detergent fiber in order to provide a basis for comparing present results with older data. The relationship is:  $\text{crude fiber} = 3.56 + 0.75 \times \text{acid-detergent fiber}$ . (AH h2-6)

(b) Effect of heating and drying of forages and feces on analyses. Other studies have been made comparing the effects of heating and drying upon the yield of acid-detergent fiber and lignin. Data show that the acid-detergent fiber from unheated forages contains 2-20 percent of the total plant nitrogen. This nitrogen has a low in vivo digestibility and is associated with the lignin fraction. When forages are heated or dried, the fraction insoluble in 72%  $\text{H}_2\text{SO}_4$  tends to increase along with the nitrogen content at a ratio of 9.16 of lignin dry matter to nitrogen. Evidence suggests the condensation of carbohydrate residues with amino acids in the non-enzymic browning reaction to form dark colored acid-insoluble polymers. Corrections have been devised for the analysis of feed materials which have been excessively heated in order to estimate the true lignin content. (AH h2-6)

(c) Estimation of digestibility using lignin ratio technique. Previous studies have shown that lignin, using previously published methods, gave variable results when used as a marker in the ratio technique for determining digestibility.

Studies have been made using the new detergent lignin as a measurer of digestibility using the ratio technique. A comparison with chromium oxide in 22 total collection trials with cows gave a correlation of +0.96 between digestibility estimated by lignin ratio compared with total collection,

whereas the corresponding correlation for chromium oxide was +0.91. Recoveries of both detergent lignin and chromium oxide were close to 100 percent. Thus the new detergent lignin procedure would appear useful in pasture studies and other experiments involving digestibility by ratio techniques. (AH h2-6)

(d) Relationship of forage composition to voluntary intake. A study of the lignin and detergent fiber contents of 83 forages from West Virginia representing 7 species has been made. These forages are from digestibility and voluntary intake trials conducted after the manner of Crampton using nutritive value index. An analysis of covariance showed that the relation between intake and digestibility is not consistent and may be a species characteristic such as lignin is known to be. Neither acid-detergent fiber, nor detergent lignin, were good predictors of intake, and other analyses for this purpose should be sought. Consequently, the attempt to predict nutritive value index directly from laboratory analyses is not considered feasible. (AH h2-6)

9. Influences of high protein on the heart rate of dairy cows. Data collected at Beltsville and elsewhere indicate that the maintenance requirement for a grazing cow is greater than for the same animal stanchioned in the barn. It had been suggested that this requirement could be due to increased intake of protein with a resulting increase in heat increment. Two groups of cows were fed a pelleted ration containing 26 and 10% protein in a reversal experiment. Heart rates were taken as a measure for possible increased heat production. No difference was detected between the two groups. It was concluded that increased protein intake per se is not the major factor concerned with increased maintenance requirement of grazing animals. (AH h2-8)

#### 10. Chemicals in milk.

(a) Effectiveness of hydrobiotite on in vivo removal of radionuclides from milk. A pilot study to determine the effectiveness of hydrobiotite (Vermiculite) in the ration on the excretion pattern of radionuclides ( $\text{Cs}^{134}$ ,  $\text{Ba}^{140}$ ,  $\text{Sr}^{85}$ ,  $\text{Ca}^{47}$ ,  $\text{I}^{131}$ ) was conducted. The inclusion of hydrobiotite at the rate of 1.5 lbs. per day (3% of the total ration) resulted in a marked reduction of radioactive cesium, barium, and strontium in the milk. In vitro and in vivo tests with  $\text{I}^{131}$  were negative for all grades of hydrobiotite tested. One unexplained observation made in the pilot studies was that the hydrobiotite apparently caused mobilization of bone and tissue stores of  $\text{Sr}^{85}$ , as evidenced by an initial increase in the  $\text{Sr}^{85}$  in the milk followed by a more rapid decline in concentration than in the control period. Two cows and eight laying hens were used in the pilot studies, and replications showed that hydrobiotite is effective in influencing the excretion pattern and metabolic fate of these cations. (AH h2-8) (Also see Area 1)



(b) Concentration of thiodan in milk from residues on sprayed Coastal Bermuda grass. Coastal Bermuda grass was sprayed with 4, 8, and 16 ounces of thiodan per acre. The Bermuda grass was harvested 7 days later and placed in tower silos with the addition of 100 lbs. of ground ear corn per ton of forage as an aid to preservation of the silage. The silage was fed out beginning 78 days later to lactating dairy cows over a period of 3 weeks. During this storage period about 2/3 of the thiodan disappeared from the silage. The silage as fed contained 1.24, 1.89, and 6.43 ppm of thiodan on a dry matter basis for the 4, 8, and 16 ounce rates of spraying. No thiodan residues were found in the milk. Therefore, it is concluded that forage sprayed with thiodan and ensiled does not result in thiodan residues appearing in the milk. Considerable thiodan disappears from the ensiled forage during storage in the silo. (AH h2-9)

## B. Forage Evaluation and Utilization

### 1. Silages

#### (a) Storage structures

(1) Storage of low-moisture silage in conventional tower silos. Excellent quality high dry matter silage was previously produced in conventional tower silos by following somewhat unusual filling and sealing procedures. This experiment was extended by investigating whether an excellent product could be obtained by more simplified procedures.

First-cutting alfalfa was mowed, conditioned, and harvested as either barn-dried hay or as low-moisture silage (about 45% dry matter) stored in two upright silos. The usual good filling procedures were followed and distribution in the silo was limited to occasional manual leveling in one silo and mechanical distribution in the other. This resulted in 88 and 92% dry matter preservation, low ammoniacal N production, and a limited fermentation predominantly lactic acid. The low temperatures observed and considerable residual sugar were also characteristic of a well-controlled atmosphere. The feeding value of these silages for milk cows and heifers and digestibility for sheep was equal to the companion hay.

The results were similar in all respect to those of the previous year. Thus, it appears that low-moisture silage can be successfully made in conventional tower silos with the application of the usual good ensiling practices. This means that the weather losses and hand labor associated with hay making can be economically eliminated or reduced by most farmers. (AH h3-3)

(2) Storage of low-moisture alfalfa in bunker silos. Successful storage of low-moisture alfalfa in conventional tower silos and wilted alfalfa in a bunker silo suggested that alfalfa at about 50% dry matter might be stored in a bunker.

Second-cutting alfalfa in full bloom was mowed, conditioned, wilted to an average dry matter content of 47% (range 24-70), chopped short, and stored in a bunker. Both bunker walls were lined with plastic and the excess on one side was put under the top seal (envelope seal) while the excess on the other wall was placed on top of the top cover (overlap seal).

More than 90% of this forage was recovered for winter feeding. Visible spoilage losses were 3.6% and occurred mainly on the side of the silo with the overlap seal. Maximum temperatures of 105°F were observed soon after filling. The chemical quality was excellent and typical of well-preserved haylage. No critical evaluation of feeding value was made.

The storage of low-moisture alfalfa silage in a bunker was clearly successful. This work should be extended to fully evaluate the possibilities of producing the best silage (high dry matter) with a minimum silo investment. (AH h3-3)

(b) Factors affecting silage quality

(1) Effect of pre-ensiling air exposure on quality of orchardgrass silage. Field wilting of forage involves exposure of the crop to air as well as a reduction of moisture content. While the overall effect is usually an improved silage, the effect of exposure has not been distinguished from that of moisture reduction.

Third-cutting orchardgrass, both chopped and ground, was exposed to air from 0-8 hours before being ensiled in one quart silos. Restricted circulation during this period minimized moisture loss. This type and length of exposure had no significant effect on silage from the ground forage, but a marked deleterious effect on chopped forage exposed for 8 hours was noted.

These results suggest that the benefits derived from fast wilting may be greater than those from slow wilting. If true, this might be of considerable importance when attempting to wilt to a 50% moisture level. (AH h3-3)

(2) Effect of antibiotics on orchardgrass silage. Interest in improving the fermentation pattern of high-moisture direct-cut silage continues. Tylosin and zinc bacitracin treated silages were compared to a control silage using third-cutting, nitrogen fertilized orchardgrass ensiled in steel 4' x 8' silos. No beneficial effects of either antibiotic were noted since all silages, including the control, were of good quality. Reliable methods of producing poor quality control silages for such experiments are needed. (AH h3-3)

(3) Effects of various mechanical treatments on specific gravity, losses and quality of orchardgrass silage. A distinct improvement in chemical quality of silage has been noted from fine grinding forage previous to ensiling in quart jars. The effects of grinding, very fine chopping, and laceration were compared with respect to the



improvement of fermentation over that achieved by regular chopping. Fine chopping, laceration, and grinding produced increasingly greater specific gravity as compared to chopping. pH, ammoniacal nitrogen, and acetic acid after ensiling were significantly negatively correlated with specific gravity before ensiling. Thus, the beneficial effect of mechanical treatments on fermentation can be assessed by measurements of increases in specific gravity of the forage caused by the treatment. Dry matter loss and CO<sub>2</sub> loss was also favorably affected by increasingly severe mechanical treatments. (AH h3-3)

(4) Effect of laceration on preservation and feeding value of grass-legume forage. This work is an extension of laboratory studies, which had shown beneficial effects from laceration on silage quality, to a farm silo situation. Lacerated and non-lacerated forages were compared in farm size silos at Lewisburg, Tennessee. This report gives results for the second year of study. Some small differences in favor of the lacerated silage in chemical quality were noted. For instance, the pH of the control was 4.36 compared to 4.03 for the lacerated. In the same order the butyric acid was 1.25 vs. 0.29%, lactic acid 6.5 vs. 7.7%, ammonia nitrogen as protein 2.8 vs. 1.8. There were no differences in feeding value in terms of dry matter intake or milk production. These results agree with those obtained in the previous year. It can be concluded that laceration of forage has no advantages where the forage is stored in a tower silo. It seems probable that the weight and pressure of the forage probably produces a "laceration effect" automatically in a tower silo. The possible advantages of laceration of forage to be stored in horizontal silos where pressure is not so great should be investigated. (AH h3-3)

(5) Effect of nitrogen fertilization on silage quality. The variation from year to year in silage quality which results from ensiling an apparently similar crop by the same methods is a matter of concern to both researchers and farmers. The role of crop fertilization in creating these differences was investigated, using first-cutting orchardgrass. Grass fertilized with 400 lbs. of ammonium nitrate on April 27 was direct-cut and ensiled in comparison to the same crop without nitrogen fertilization. The fertilized forage contained 23% crude protein when harvested at full-head stage (May 15-17) and the unfertilized contained 13%. The fertilized forage was also lower in dry matter, crude fiber, and sugar content.

Silage from the fertilized crop was higher in ammoniacal nitrogen, butyric acid, and pH. The poorer quality was also evidenced by lower consumption by dairy cows and by weight loss. The effect of nitrogen fertilization on silage consumption was more marked than it was in a previous experiment. This is explained to a large extent by the greater effect of nitrogen on the chemical composition of the silage in the present experiment.

The effects of fertilization were further studied in 4' x 8' silos. In this study 800 lbs. of ammonium nitrate per acre produced even greater changes in plant composition and deleterious effects on fermentation products, particularly between the first and eighth week of storage. Initial nitrate contents

of 1 - 1.7% were observed in the 400 and 800 lb. fertilization treatments but were reduced to near zero during 8 weeks of storage. Sodium nitrate added at the time of ensiling had little effect on the chemical quality of control silage. The added nitrate remained at a high level in the control silage. This has since become regarded as typical of nitrate in a low pH silage. The poor chemical quality produced by fertilization did not display high counts of anaerobic spore formers as was the case when poor quality was produced by aeration.

The production of poor quality silage by nitrogen fertilization of the crop suggests that the practice should be combined with the use of silage preservatives or wilting. Lowered silage intake rather than toxicity has been the prominent hazard in these highly fertilized silages. (AH h3-3)

(c) Yield and feeding value of silages

(1) RS-610 grain sorghum and starr millet as silage. At Lewisburg, Tennessee, the yield, preservation, and feeding value of 610 grain sorghum and starr millet were compared when harvested as silage. The 610 grain sorghum yielded 4 tons of dry matter while the millet yielded 3 tons of dry matter per acre. 88.7 and 97.4% of the dry matter, respectively, was preserved as silage for feeding. Milk production in the same order was 37.7 and 34.2 FCM daily. The first year's data show a considerable difference in yield for the two crops but no significant difference in feeding value. (AH h3-12)

(2) Relative feeding value of four silages. At Willard, North Carolina, four different crops were harvested as silage. The four crops were mature corn, drilled immature corn, Hegari sorghum, and Gahi millet. The drilled immature corn had 232 lbs. of ground ear corn added at the time of ensiling, which amounted to 36.1% of the dry matter of the silage. The relative feeding value of the four silages was determined in trials with lactating cows and growing heifers and in a metabolism trial with steers.

The dry matter intakes of immature corn and Hegari sorghum by the heifers were similar, and were significantly higher than those observed for mature corn and millet. However, the lower intake of mature corn was not reflected in average daily gain with only the gain on millet being significantly inferior.

In the lactation trial, dry matter intakes of Hegari sorghum were higher than those for the other silages. Intakes of mature and immature corn were similar, with millet significantly lower. FCM production was similar for all silages except millet, which was significantly lower. Weight gains on Hegari appeared to reflect the higher silage intake, but were not significantly different from the others.



No significant differences were noted for the dry matter or crude protein digestibility or for nitrogen retention by the steers in the metabolism trial.

Thus, millet silage was markedly inferior to the other three in both growth and lactation trials. Although immature corn compared favorably in feeding value with mature corn and Hegari, probably one-third of the dry matter fed out came from the ground ear-corn additive. (AH h3-9)

(3) Yield of sorghum silages. In the southeast area of the United States, because of the relative uncertainty of corn as a silage crop, as a result of occasional drought conditions, considerable attention is now being given to the value of sorghums as a silage crop. At Willard, North Carolina, the relative yield of Sart and Hegari sorghums were compared to corn for silage.

The yield of Sart sorghum, Hegari sorghum, and corn were 6.04, 13.0, and 5.6 tons of dry matter per acre.

Thus, although wet yields of Sart exceeded those of corn, dry matter yields were essentially the same. Hegari yields were poorer in previous years, but some additional forage was obtained in a second cutting.

The plant separation data confirms the high stalk percentage and low grain proportion of Sart compared with corn and Hegari. It also points out the possible effects of seeding and harvest time on plant composition, even though the stage of plant at harvest was similar.

Feeding trials are in progress to evaluate these silages and to see if production of animals fed Sart can be made to equal corn with additional concentrate feeding. Two plastic bag silos of Sart with ground corn cobs added to one as a preservative will also be compared. (AH h3-8)

(4) Utilization of silage protein. Two silages from the same field containing 25.6 and 14.4% crude protein were fed to sheep in digestibility trials. The high protein silage was produced by fertilization with 400 lb. of ammonium nitrate per acre. The animals on the control silage (no nitrogen fertilization) showed positive nitrogen retentions although the ration was deficient in energy and provided only a borderline level of intake in relation to requirements. The sheep on the high nitrogen silage retained 94% more nitrogen than when on the control silage. The amount of protein retained for the two silages was 39 grams for the high protein silage and 17 grams for the control. While it is well known that much of the nitrogen in silage is non-protein nitrogen and is in the form of ammonia, amide and amine nitrogen, yet the sheep in this experiment on the control ration utilized the nitrogen efficiently. (AH h3-3)

(d) Biochemical studies relating to silage investigations

(1) Determination of nitrate in silages. The development of a chemical method that will result in reliable values for the nitrate content of plant material has been needed for several years. A method has now been worked out, data summarized, and a paper prepared for publication.

Better recovery of nitrate from charcoal - Celite columns has been found with phosphoric acid rather than sulfuric acid in the initial eluent. Phosphoric acid, on the other hand, was found to be less effective than dilute sulfuric acid in extraction of nitrate from plant material. However, dilute phosphoric acid containing 4% of sodium sulfate proved fully as effective as dilute sulfuric acid for extraction. In the elution of nitrate from charcoal columns it was found that the concentration of sodium bicarbonate in the eluent could be reduced from 1.0 to 0.1%. The reduced level of bicarbonate resulted in smaller dried salt residue and consequently speedier solution by the nitration solvent. Nitrite interference was found to be easily eliminated by addition of sulfanilic acid to the nitrate solution before chromatography. (AH h3-1)

(2) Determination of sugars. The need for the development of a stoichiometric colorimetric method for the determination of sugars has been widely recognized. The use of toluidine in acetic acid as a reagent to give equal response per molecule of sugar has been investigated. The effect of borax as a catalyst in the reaction of the reagent gave an equivalent response for glucose and sucrose. Further study showed that for lactose, marmose, and xylose, the respective ratios to glucose were 1.04, 0.98, and 1.16 which is essentially within the experimental area of exact agreement. Galactose, however, gave an aberrantly high response which was not encountered with lactose. Treatment of the final reaction brought the ratio for glactose into line with the other sugars. Further tests are in progress for pentoses, methyl pentoses, and various other sugars to study the general applicability of the reaction. Present data indicate that the reaction may not yield a stoichiometric method only for mixtures of aldoses but also for ketoses. A further advantage for the reagent is that the disaccharides may be determined directly without prior hydrolysis. (AH h3-1)

(3) Nitrate disappearance in silage. The nitrate content of hay-crop silage decreases during the early stages of fermentation and subsequent storage in the silo. The factors that affect the degree and rate of decrease are not well understood. In experiments to study those factors, the nitrate level of orchardgrass was followed through the ensiling process in 10' x 35', 4' x 8', and quart jar silos. The results showed that a high pH type of fermentation that takes place in high-moisture silage will almost completely remove nitrate from the forage. Wilting the forage in the field prior to ensiling tends to prevent nitrate disappearance from the silage. The addition of sugar to high-moisture forage tends to prevent nitrate disappearance. Grinding or masceration of the forage facilitates the removal of nitrate in the early stages of the fermentation.



As a result of these studies, it has been suggested that nitrate removal from direct-cut silage may take place in two stages in the fermentation. Some disappears shortly after the silage is placed in the silo before the pH becomes lowered. Laceration hastens disappearance at this time and may result in almost complete removal. Then after a period of storage more nitrate disappears if the pH for some reason becomes elevated. (AH h3-1)

(4) Effect of herbicides on the quality of silage. It has been known for some time that the treatment of crops with herbicides will result in an increase in sugar content of the crop. It is also known that the sugar content of forage at the time of ensiling improves the quality of the silage produced due to the production of a desirable type of fermentation. Therefore, it was proposed to study the effect of spraying forages with herbicides on the quality of the resulting silage. Alfalfa was sprayed with 2 different herbicides and orchardgrass was sprayed with 3 other herbicides and the resulting forage placed in quart jar silos 0, 1, 2, and 4 days after spraying. The lactic acid content of the resulting silage was used as an index of quality. The results showed that some of the herbicides definitely increased the lactic acid content of the silage, the greatest effect usually occurring on the second and fourth day after spraying. The results were most encouraging and suggest that further study is highly desirable. (AH h3-1)

## 2. Pastures

(a) Stocking rate of dairy cows on orchardgrass ladino clover pasture. A study of the effects of intensive, moderate, and liberal grazing pressure on rotationally grazed pastures on the productiveness of the pastures and milk production by dairy cows has been continued at Beltsville for a third year which completes the experiment. Effects on cows, plants, and acre yields were obtained in cooperation with the Forage Crops Branch.

The data from tester cows indicated that the digestibility of forage was lowered slightly but significantly by more intensive grazing. Digestibility was significantly higher at the beginning of the grazing season. Intakes of dry matter, digestible dry matter, organic matter, and digestible organic matter were lowered by intensive grazing to a small and insignificant extent. These findings were in accord with the finding that levels of milk and FCM production were not significantly different between treatments.

Further interpretation of the data, including effects on production per acre of forage and animal products, is underway. (AH h3-18)

(b) Coastal Bermuda grass for lactating dairy cows. At Willard, North Carolina, in the coastal plains area, permanent type pastures do not produce well. Because of this, considerable attention has been given to the use of annual crops in the pasture program. However, because Coastal Bermuda

grass has increased in popularity as a permanent type of pasture for beef cattle, it was thought desirable to study the use of the grass with dairy cattle. A comparison was made between rotationally grazed Coastal Bermuda grass, rotationally grazed Starr Millet and dry lot feeding of corn silage as the forage. Results for the second year of the study are reported. Jersey cows produced 28.1, 31.1, and 28.4 lbs. of FCM per day on the Coastal Bermuda grass, Starr Millet, and corn silage, respectively. Percent decline for a four-week period was 10.9, 10.0, and 7.9%, respectively. Gain or loss of body weight was -0.03, -0.03, and +0.29, respectively. For this year Starr Millet was superior for milk production. (AH h3-10).

(c) Stored forage compared to rotational grazing for lactation. It has been suggested with the increased development of silage harvesting equipment and automatic silage unloaders that there might be some advantage to harvesting the forage rather than pasturing it. At Lewisburg, Tennessee, a comparison of this type has been in progress using a field seeded to orchardgrass, ladino clover, and alfalfa. At the end of 3 years the plant population for the stored forage and pasture plots were alfalfa 34.1 vs. 16.7%, orchardgrass 35.5 vs. 45.5%, ladino clover 25.2 vs. 28.1%, and weeds 5.3 vs. 9.7%. Milk production per cow has been in favor of the pastured animals while milk production per acre has been slightly in favor of the stored forage fed cows. (AH h3-12)

3. Relative feeding value of wafered and baled alfalfa. Harvesting and feeding of hay in the form of wafers have attracted considerable attention recently. This interest has been based primarily on the physical advantages of handling hay in this form while the nutritional status of wafers has been less clear.

Field-cured hay was field cured and then baled or wafered with a Ford machine. Milking cows consumed more dry matter and produced more milk when fed the wafers. The wafers contained less crude fiber and more crude protein when fed. This is suggestive of less leaf loss in the wafering process. From this limited trial it appears that any nutritional advantage of wafers over baled hay may lie in differential mechanical losses and/or greater ad lib intake. (AH h3-3)

## C. Calf and Heifer Feeding

1. Rumen content relationships with silage and hay rations. In previous experiments the preservation of forage as direct-cut silage has resulted in lower dry matter consumption and lower weight gains by dairy heifers than results from feeding hay. The storage of forage as direct-cut silage has usually resulted in a decrease in both the content and solubility of carbohydrate and an increase in both the content and solubility of the nitrogen. It might be expected that if there was an increase in acid-detergent fiber in direct-cut silage as compared to hay, it would reduce the rate of digestion and the rate of disappearance from the rumen and, therefore, be a major factor in differences in intake.



The current experiments were undertaken to determine the relationship of the feed composition, digestibility, and passage from the rumen to feed intake of direct-cut silage and hay harvested at the same time from similar areas. Secondly, these experiments have explored the effects of lower nitrogen utilization observed when feeding direct-cut silage compared to hay on growth rates of dairy heifers. In this experiment the preservation of forage as high-moisture silage increased the lignin, acid-detergent fiber, total Kjeldahl nitrogen, and ammoniacal nitrogen compared to preservation as hay. The silage showed reduced dry matter and organic matter digestibilities but equal energy and nitrogen digestibilities.

The hay and silage was fed at maintenance and ad libitum levels of intake. Ad libitum hay dry matter intake was significantly higher than ad libitum silage dry matter intake. When the entire rumen content was removed at certain hours after feeding, the highest level of both wet or dry rumen content was observed on the ad libitum hay ration. Identical rumen retention times were observed for ad libitum silage and hay. Feeding hay or silage restricted to maintenance intake gave rumen retention times similar to each other but somewhat greater than ad libitum feeding. Adjusting the rumen retention time on ad libitum silage for the effect of the slightly lower consumption observed on ad libitum hay would tend to give it a shorter retention time than observed for hay. Dry matter disappearance measured by direct removal from the rumen which measures the sum of the two rates of disappearance, i.e., digestion with absorption from the rumen and passage down the tract, was not significantly different for hay and silage rations. These data suggest that accumulation of rumen residues or rumen capacity was not limiting the dry matter intake of this silage ration.

Ration effects on percentage dry matter in the rumen occurred. These resulted, in part, from the differences in level of feed intake. Ad libitum feeding increased the percentage dry matter in rumen compared to maintenance level feeding. Animal differences existed for the percentage dry matter in the rumen. Wet rumen content and dry rumen content decreased with time after feeding. No differences appeared in percentage dry matter of ad lib fed animals.

The silage contained 3.40% total nitrogen in the dry matter with 27% of the total as ammoniacal nitrogen and 23% as hot water insoluble (true protein) nitrogen. The hay contained 2.52% total nitrogen in the dry matter with 2% of the total as ammoniacal nitrogen and 61% as hot water insoluble nitrogen. The nitrogen utilization on hay was better than on silage when both were fed ad lib. This is true whether basing the comparison on grams of nitrogen retained per day, percent nitrogen retained of that fed, or percent nitrogen retained of that digested.

A second group of animals were fed continuously to measure intake and weight gain. The hay fed animals consumed more dry matter and gained more weight.

The growth of these animals and the nitrogen retention of those used in the nitrogen balance trial were combined to further study nitrogen utilization using a different concept than nitrogen retained per day as previously pointed out. In the second concept the grams of nitrogen retained per 100 grams of body weight gain were calculated. The results for silage were much above those for hay and much above those usually accepted for adequate protein supplements. The large amount of nitrogen retained per 100 gm. gain on silage suggests in contrast that body weight gain was not restricted by the lower utilization of silage nitrogen. The large amount of nitrogen retained per unit of weight gain for the silage fed animals in contrast to the lower utilization of nitrogen expressed as grams retained per day suggest that energy intake is the primary limiting factor causing lower weight gains in silage fed animals. (AH hl-1)

2. Effect of frequency of feeding milk replacer to calves. Some published data indicated the greater frequency of feeding may increase rate of gain. Calves were fed milk replacers at two levels of intake and at 2 or 4 times each day. Results obtained at Willard, North Carolina, with 12 Jersey calves showed greater gain resulted from the higher level of feeding but there was no difference in gain due to the number of times the calves were fed each day. (AH h3-11)

#### D. Management Practices, Equipment and Facilities.

##### 1. Bovine mastitis.

(a) Effectiveness of antistaphylococcal vaccination. A study was made of the efficacy of antistaphylococcal vaccination in controlling mastitis. In May 1961, the station veterinarian began a program of vaccination employing a commercial multi-strain Staphylococcus aureus mixed bacterin-toxoid, Jen-Sal "Staphoid A-B", consisting of beta-propiolactone-inactivated whole cultures, among which were representatives of phage groups I, III, and IV. All cows and bred heifers were paired according to breed, age, and stage of lactation and assigned to either the treated or control group. Heifers were added alternately to the experimental groups as they became pregnant. Vaccination was carried out according to the manufacturer's recommendations.

During the succeeding year and a half the vaccinated and control groups exhibited, respectively, 41 vs. 38 chronic cases of mastitis; 17 vs. 13 acute cases. On a per-quarter basis, there were 46 vs. 39 chronic and 20 vs. 13 acute. The groups required, respectively, 204 vs. 174 treatment days. Since March 1962, when facilities for bacteriological analyses became available, the herd has been subjected to monthly quarter sampling of strict fore-milk. The udder microflora was characterized quantitatively and qualitatively, and leucocyte concentration was determined. Laboratory findings of infections caused by coagulase-positive staphylococci among the treated and control groups indicate no influence of vaccination on the incidence of



infection or on its elimination. The respective frequencies were: 25.6% vs. 31.0% of cows infected, and 12.8% vs. 10.7% on a per-quarter basis; 5.7 vs. 16.1% of heifers infected, and 2.2% vs. 6.3% on a per-quarter basis. The numbers of S. aureus infections occurring among the primips were so low that, although the percentages show an interesting difference in favor of the vaccinated animals, no significance is attached to them. Mean concentrations of leucocytes (per million) were as follows for the vaccinated and control groups, respectively: 0.63 vs. 0.73 among quarters free of culturable bacteria; 4.95 vs. 4.08 among quarters positive for S. aureus.

These results show no benefits accruing from vaccination of this herd with the bacterin-toxoid. There was no difference between the groups of animals in (a) frequency of S. aureus infections, (b) incidence of clinical mastitis, or (c) severity of staphylococcal mastitis.

(b) Factors influencing leucocytes in milk. Five thousand quarter milk samples were assayed for leucocyte concentration by direct microscopic count. An estimation of reliability of counting procedure was made using a 10 field count as compared to a 27 field count. Repeatability of the 10 field count using 6 cows and 5 slides from each quarter was 0.965. The corresponding value for the 27 field count was 0.981. The ten field count was then used because of its advantage in greater speed.

Data obtained from monthly quarter samplings were analyzed in terms of leucocyte count distributions among quarters classified as uninfected or infected. The majority of bacteriologically negative quarters had leucocyte counts of less than 500,000 per ml. However, 16.6% of these counts were greater than 1,000,000 per ml.

Among quarters infected with S. aureus only 10% of the leucocyte counts fell below 1,000,000 per ml. If dairy processing plants set maximum normal counts at 200,000 per ml to indicate abnormal milk (as is being proposed by some) the then 55.9% of the culturally negative samples would have been misclassified and rejected. Of the samples positive for S. aureus, 5.7% would have been accepted. If the grading limit was set at 1,000,000 per ml., then 16.6% of the negative samples would have been rejected and 9.2% of the S. aureus samples would have been accepted.

The reasons for the large number of negative samples having high leucocyte counts could be as follows: (a) lingering inflammatory response to recent infection, (b) traumatic inflammation of non-microbial origin, (c) current infection, which was detectable culturally, or (d) other factors not readily understood. To further understand these results an analysis was made on a group of selected cows having consistently uninfected quarters. This study suggests that high leucocyte values do not reflect recent history of udder infection but to some extent, the individuality of the cow and even the quarter. Age of the animal, lactation number and stage of lactation seem to be important in the pattern of leucocyte shedding. (AH g3-8)

2. Environmental influences affecting production records. This project was initiated to develop methods for minimizing the environmental influences on production records and thus improve the records as estimates of genetic merit. The work is carried out at Beltsville, jointly with the Dairy Herd Improvement staff of the Dairy Cattle Research Branch and in cooperation with the Wisconsin Agricultural Experiment Station.

(a) Environmental variables associated with milk yield. Feeding and management data collected by fieldmen and DHIA supervisors were analyzed by simple correlation and multiple regression procedures in an attempt to determine the independent influence of management and feeding variables on herd level of production. After a series of multiple regression analyses it was found that 10 of the 53 environmental variables had important relationship to herd average milk yield. These variables were: 1. cow cleanliness; 2. recommended machine line vacuum; 3. recommended pulsation; 4. number additional helpers at milking; 5. calf feeding; 6. herdsmanship; 7. hay score; 8. percent days in milk; 9. herd size; and 10. TDN/1000 pounds weight. The multiple coefficient of determination from this analysis indicates that 65% of the variation in yearly herd average milk yield was associated with variation in these 10 environmental influences.

In each of 5 successive multiple regression analysis in which a single additional independent variable was removed from the study until in the 5th analysis only 5 independent variables remained, it was found that as variables numbered 4, 5, 1, 10, and 6 were removed the respective  $R^2$  values found were .62, .60, .60, .58, and .43. The procedure simply eliminated, stepwise, the 3 subjectively recorded ratings, variable No. 4, which has questionable meaning and variable No. 10 which has questionable cause and effect relationships.

(b) Variations in tape weight. From 13,723 tape measurements, representing 4, 595 cows in the environmental project herds, heritability and repeatability of lactation weight were estimated from the first through sixth lactations. Heritability estimates were derived from paternal half-sib correlations. Those estimates obtained from an overall herd basis were 0.23, 0.53, 0.41, 0.79, 0.79, and 0.64, for the first through the sixth lactations, while the corresponding values obtained from a within herd basis were 0.31, 0.53, 0.76, 0.85, 0.14, and 0.40. These estimates were somewhat greater than most of those previously reported for live weight.

Repeatability estimates were high and ranged from 0.62 to 0.77, when obtained from the between cow analysis of variance; 0.61 to 0.78 when obtained from a between sire analysis; and 0.57 to 0.74 when obtained from the between herd analysis of variance. These values were similar for each lactation.

These results were not conclusive in determining which single month would be most reliable in estimating lactation weight. It was apparent that the first



month of lactation was the least reliable month to obtain average lactation tape-weight because both the tape and scale weight overestimates average lactation tape-weight and because appreciably greater variation in tape-weight existed during the first month of lactation.

(c) Variations in DHIA test-day production: The monthly test-day yield involving 4,167 lactation records made in the environmental project herds from September 1958 to October 1962 were evaluated in studying variations in test-day production involving DHIA herds. Each test-day production, including the first, was expressed as a percentage of that of the previous month and the effect of age at calving, season, and year of calving, and level of production on these values, was determined by a least squares analysis of variance. Age was highly significant in its effect on test-day changes in fat production, while season of calving showed significance only in early and late stages of lactation. Year and level of production had no significant influence on test-day changes in fat yield.

Test-day change figures for each month appeared to have a normal distribution about their means, with the possible exception of the last month of lactation. The amount of variation associated with test-day change varied with month of lactation. The 2nd, 9th, and 10th months had the greatest variability, and months No. 4, 5, and 6 were least variable.

Nurse cow tables were derived, based on the average lactation curves for test-day milk yield. It was concluded that the highly significant effect of age on test-day changes could be adequately accounted for by having two nurse cow tables, one representing ages of less than 36 months and the second ages greater than 35 months.

The results of this study indicated that DHIA processing centers should give serious consideration to automating the adjustment of production credits of cows assumed to be abnormal at approximately the one percent level of probability. This would be considerably less than the 2.87% which could be adjusted based on the existing 40% DHIA rule. It was further suggested that such adjustments be made without regard to indicated abnormality remarks by the DHIA supervisor, since these results showed that in Wisconsin DHIA such remarks are highly selective.

(d) Evaluation of herd averages. The production data collected from the environmental project herds between November 1958 and January 1962 were used in evaluating variations in different methods of computing DHIA herd averages. It was found that in computing DHIA herd average production the adjustment for back credit is unimportant in the monthly and in the current month of the twelve-month rolling DHIA averages.

Monthly and twelve-month rolling averages for milk and fat yield were regressed on the corresponding percent days in milk, stage of lactation, herd size, and herd age. These variables accounted for approximately 39, 30, .1,

and 0 percent of the variations, respectively, in monthly milk and fat yield. It was concluded that percent days in milk and stage of lactation were important sources of variation in monthly production averages. A similar analysis involving twelve-month rolling herd averages indicated that stage of lactation was an important source of variation. It was estimated that 12 and 6% of the variation in monthly milk and fat yield, respectively, was attributed to year, season, and year x season interactions. Year x season interactions were highly significant for monthly milk and fat yield and stage of lactation. Year and season influences were highly significant sources of variation in percent days in milk.

Each of ten months of computing herd averages from lactation data was compared to the corresponding twelve-month rolling DHIA herd averages for the 46 herds over 3 years. It was found that M.E. extended lactation records based on all available lactations were at least as adequate as 9 other methods of defining herd averages based on lactation records in predicting the level and in ranking herds according to the DHIA rolling herd average. The multiplicative factor derived from these data in attempting to convert lactation averages to actual or DHIA rolling averages was 1.06. It was apparent, however, that relatively large errors would be made in using this factor on an individual herd basis.

(e) Selection of females. During the years 1959-1960 and 1961, a total of 5,218 calves were born in the project herds. Of these, 48.0% were heifers, 48.5% were bulls, and 3.5% were not identified as to sex. An inventory of 794 heifers born in 1959 and compiled 2-1/2 years later indicates that, from 45 to 50% of the heifers born in DHIA herds such as those in the environmental project, eventually freshen into the herd.

An attempt was made to characterize the project herds as to why cows in milk leave the herd and the extent to which their removal is associated with production levels. The 41% leaving the herd because of culling or selection were significantly inferior to their herd mates by approximately 1,100 lbs. of milk and 50 lbs. of fat. This level of inferiority, or culling effectiveness, was similarly evident both when all available and first records were involved in the analysis. The production inferiority of cows culled from the herd for reasons other than low production was considerably less and not highly significantly different from that of their herd mates. These results strongly indicate that in the herds enrolled in the environmental project, some degree of effectiveness has been realized in the selection or culling of milking females. It was also apparent that cows eventually to be culled from herds could be effectively evaluated and culled on the basis of their first records. (AH g5-1)

3. Antibodies in milk. The purpose of this work is to determine if absorption and/or protection occurs when milk containing antibodies is ingested by humans or livestock.



The udders of 10 cows were infused with bacteria (heat-killed, phenolized Escherichia coli - Norden Strain 158 or Proteus mirabilis) at weekly intervals from 250 days of gestation to parturition. Each quarter received a suspension of  $5 \times 10^9$  bacteria in 25 ml. of sterile saline. A rise in antibody titer occurred in the blood and milk. Milk titers were highest in colostrum with peaks as high as 262,000. It has been reported that antibody milk protected calves against lethal oral doses of E. coli (Norden Strain 158). However, in the present study, oral doses of up to  $465 \times 10^9$  bacteria failed to cause ill effects in 11 calves ranging from 5 to 38 days of age. Studies on 8 one-month-old calves indicated that intravenous doses of  $7 \times 10^9$  E. coli were lethal. Nine pairs of one-month-old calves were challenged with  $7 \times 10^9$  live E. coli intravenously after one of each pair consumed 14 lbs. of antibody milk per day for five days. Antibodies could not be detected in the blood of these calves after drinking the milk. Two of nine calves drinking the antibody milk survived. One control calf survived. Ten adult human males drank a quart of antibody milk a day, a pint at a time, for six days. Tests of blood samples taken before and after the milk was consumed failed to indicate that antibodies had passed into the blood. These studies failed to confirm the reported passage of antibodies into the blood after ingestion of milk containing them. Also, there was no indication that milk from cows infused with E. coli could protect calves from a minimum lethal dose of the bacteria given intravenously. (AH gl-8)

4. Physical methods of fly control. These investigations were initiated to evaluate and develop equipment and physical methods for the control of flies and other livestock pests. The ultimate objective is to reduce or eliminate the use of agricultural chemicals around products designed for human consumption. This work is cooperative between Agricultural Engineering, Entomology, and Animal Husbandry Research Divisions.

Preliminary studies conducted in temporary facilities at Orlando, Florida, and at Beltsville, indicated that "Black Light" ultraviolet radiation is attractive to both house flies and face flies during twilight periods. Use of fluorescent panels behind the light sources appeared to increase attractiveness.

A successful colony of face flies has been established from wild individuals. Studies are now being conducted to determine the effect of colony illumination levels on face fly egg production, period for development, adult longevity, and adult behavioral responses. Observations were made on the behavior of face flies in the field during twilight to determine characteristics which might be useful in applying controls. Face flies were marked, released, observed, and some relocated after sunset. Test equipment and techniques are being developed to evaluate the attractiveness of visible and ultraviolet radiation to face flies, house flies, and stable flies. (AH g3-12)

5. Influences of management practices and environmental factors on adaptability. These investigations involve the determination of the effect of environmental influences, including climatic elements, on dairy cattle adaptability and the evaluation of management practices on the performance of dairy cattle in hot and humid regions. The work is cooperative with the States of Georgia, Louisiana, and Texas. Some of the studies are also in cooperation with Agricultural Engineering and Animal Disease and Parasite Research Divisions. These projects contribute to the Southern Regional Dairy Cattle Breeding Project, S-49.

(a) Summer temperature patterns in the Southern United States. To provide an estimate of the frequency of occurrence of ambient temperatures high enough to affect the physiological well-being and productive performance of dairy cattle, a study was made of summer temperature conditions in 15 Southern States. The data were obtained from the U. S. Weather Bureau. Mean daily temperatures of 75°F or above for 20 or more consecutive days were considered as the point where changes in management practices to ameliorate the effects of the climatic conditions are warranted. The number of days the mean daily temperature can be expected to equal or exceed 75°F, or 80 or above and 90°F or higher was ascertained and mapped. The results indicate a mean daily temperature of 75° or above can be expected at least 20 days throughout the entirety of the 15 States considered. The only exceptions are the immediate Pacific Coastal area and areas in the Appalachians, Ozarks, and southern Rockies. The frequency of occurrence of a 75°F mean daily temperature varies from about 185 days in the extreme southern part of Texas and southern Florida, and 160 days in the vicinity of the Arizona-California border to less than 20 days in the exception areas enumerated. Throughout the major part of the area east of the New Mexico-Texas border, there are 100 days or more during the May-October period with a mean daily temperature of 75° or above, 50 days or more with a daily mean of 80° or above, but none with 90°F or above. Areas with 20 or more mean daily temperatures of 90°F or above occurred only in the southwest corner of Arizona and the bordering area of California. The results of this study may be considered as providing a general guide to or classification of the summer climate of the southern region insofar as dry bulb temperature conditions are concerned. Indications are that there is a need for further breakdowns of specific areas as humidity, terrain, prevailing wind direction, and other factors cause wide variations in local patterns and should be considered in determining the most suitable management practices for a specific area. (AH g4-3)

(b) Seasonal forage production in Louisiana. Preliminary evidence from studies at Louisiana State University indicates climatic conditions affect forage production and management. From daily evaluations of quantity and quality of forages, it was found that on the average forages were available for grazing 352 days of the year, but quality and quantity were adequate to support good milk yields for only 133 days. Of this period, only 41 days of adequate grazing were generally available from June 1st to October 31st. Indications are that good levels of production can be maintained in the area by the use of a combination of limited grazing, green chopping and silages made from the forages harvested at the proper stage of maturity. (AH g4-3)



(c) Summer temperature conditions in relation to the productive performance of lactating Jersey cows in Southeast Georgia.

Additional multiple regression analyses were made in an effort to measure the lag effect that climatic stress might have on cow performance under summer conditions. Twenty-four climatic variables consisting of daily radiation, daily hours above 80°F, daily maximum temperature, 1100 dry bulb, 1100 wet bulb, and the product of dry bulb and wet bulb at 1100 were considered in relation to milk production, feed intake, and body temperature on 0, 1, 2, and 3 days prior to the day the performance variables were obtained. With these techniques, 19.8, 25.9, and 47.7%, respectively, of the variation in milk production, feed intake and changes in body temperature could be associated with the climatic variations during the 4-day period. Of those variables studied, hours above 80°F on the third day prior to performance accounted for the major part of the variation in milk production. Maximum temperature on the day performance was observed had the greatest influence on feed intake. Hours above 80° on the day of performance had the greatest influence on rectal temperature. The degree of association between dairy cattle performance and fluctuations in climate was not greatly improved by including climatic information obtained prior to the day of performance. (AH g4-3)

(d) Roof materials for summer shades in Louisiana. At Louisiana State University studies were made during the summer of 1962 of roof temperatures and radiation load under various roof materials and their relation to some physiological responses of Holstein cows. Roof temperatures and radiation loads were lowest when metal roofing was painted with a white plastic paint (Plasticool). The painted roofs were 14.1 and 25.1°F lower than aluminum and galvanized iron roofs. Radiation loads under the shaded areas were 50 to 56% lower than for unshaded areas. Although there were differences in radiation loads under the various shades, these were not reflected in significant differences in animal responses. Cattle maintained under tree or artificial shades showed significantly lower respiration rates and skin and rectal temperatures than cattle without shade. (AH g4-3)

(e) Impact of anaplasmosis in a dairy herd. There was a serious outbreak of anaplasmosis in the dairy herd at the Iberia Livestock Experiment Station, Jeanerette, Louisiana, in 1956 following the introduction of susceptible cattle from other stations. Since there are no effective vaccines, the removal of all reactors in the herd would have been a possible solution to the outbreak, but the close proximity of other herds and the loss of irreplaceable genetic material from the breeding experiment made this impractical. Therefore, efforts were made to minimize the infection through vector control and treatment of clinical cases. In the seven year period, 1956-62, there were 32 clinical cases of anaplasmosis in the herd, 9 of which died and the remainder recovered. In this same period there were 55 sub-clinical cases. Although the major portion of the clinical cases recovered, the disease had a significant effect on production. Those cows which came down with clinical cases during lactation produced 26% less milk and 31% less milk fat than expected for that lactation period. There was also some carry over effect into subsequent lactations as those animals that had clinical attacks averaged 7% lower in milk yield than their contemporary herdmates. These animals also

left the herd approximately 1 year earlier than their contemporaries. The evidence thus far does not indicate the "carrier state" (as judged by reaction to a complement fixation test) is a serious detriment to production, although the negative animals had slightly higher lactation yields than those showing a reaction to the complement fixation test. Experiences in the Iberia Station herd show that if susceptible cattle are to be introduced into anaplasmosis areas they should be under 2 years of age and brought in during the "off vector season". Since the evidence points to the desirability of having an anaplasmosis free herd, the trend of infection in cows and heifers was appraised. In both groups the proportion of reactors increased up until 1959 but declined thereafter in the heifers and to some extent in the cows. Although the animals entering the lactating herd for the first time were largely negative, the cows constituted a continuing reservoir of infection. From December 1956 to November 1961, 126 heifers calved for the first time. Twenty of these were reactors at the time of calving and 47 became reactors after entering the milking herd. Of the latter group, 27 became reactors within six months and the remaining 20 became reactors within 2 years after entering the milking herd. An encouraging aspect is that 50 remained negative for at least one year after first calving. Indications are that it may be possible to develop a negative herd eventually, although this remains to be fully determined. (AH g4-3)

(f) Factors affecting milk composition in two Jersey herds.

Preliminary analysis of 722 complete lactations for percent fat and solids-not-fat data from Jersey cows in herds at Tifton and Reidsville, Georgia, was studied. The major portion of the variance for fat percent and solids-not-fat percent was attributable to lactation number, length of lactation, and level of milk production. Gross estimates for the variance in fat percent and solids-not-fat percent were 0.493% and 0.170%, respectively. Adjusting for lactation number, lactation length and level of milk production resulted in variance estimates of 0.182% for fat and 0.081% for solids-not-fat. Composition of the milk produced in the Tifton herd was considerably higher in fat percent and solids-not-fat than for the Reidsville herd. The level of feeding in these two herds appears to be quite different and may account for the between herd differences noted. (AH g4-3)



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INFECTIOUS AND NON-INFECTIOUS DISEASES OF CATTLE  
Animal Disease and Parasite Research Division, ARS

Problem. Losses from infectious and non-infectious diseases of cattle, other than those due to parasites, are estimated at approximately \$600 million annually. These losses materially increase costs of production and conversely decrease profits. In turn, they contribute to the cost of every purchase of meat, milk, and other cattle products to the consumer. Some of these diseases are transmissible to man. Determination and definition of the causes of cattle diseases, explorations for efficient methods of diagnosis, prevention, control, and when feasible, eradication, are the purposes of the research program.

USDA PROGRAM

The Department has a continuing long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of cattle. Research is being conducted on the diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 57.7 professional man-years. This effort is divided among sub-headings as follows:

Brucellosis, 2.3 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Universities of Maryland, Minnesota, and Wisconsin.

Vibriosis, 5.1 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreement with the New York State Veterinary College at Ithaca.

Tuberculosis, 6.6 at the National Animal Disease Laboratory, Ames, Iowa, and through two contracts with the Michigan State University, East Lansing.

Mucosal-Respiratory Disease-Complex, 5.1 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Indiana (Lafayette) and Iowa (Ames) Experiment Stations, and the Colorado State University (Fort Collins).

Mastitis, 6.2 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California, Davis.

Respiratory Disease (Shipping Fever), 5.0 at the National Animal Disease Laboratory, Ames, Iowa.

Leptospirosis, 6.0 at the National Animal Disease Laboratory, Ames, Iowa.

Infertility in Cattle, other than vibriosis and trichomoniasis, 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

Epizootic Bovine Abortion, 3.4 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California, Davis.

Foot Rot, 4.0 at the National Animal Disease Laboratory, Ames, Iowa.

Paratuberculosis (Johne's Disease), 5.0 at the National Animal Disease Laboratory, Ames, Iowa.

Keratitis (Pink Eye), 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

##### A. Brucellosis

Research workers at the National Animal Disease Laboratory (NADL), Ames, Iowa, reported the pathology of 2 bulls, naturally infected with Brucella abortus, was studied for 5 and 2 years, respectively. Serologic, bacteriologic and histopathologic examinations were correlated with the clinical signs of the disease. Seroagglutinin and semen plasma agglutinin titers persisted at diagnostic levels throughout the study, and Br. abortus was consistently isolated from semen of both bulls. At necropsy, Br. abortus was isolated from the testes, epididymides, seminal vesicles, and the ampullae of the ductus deferens. Pathologic changes were observed throughout the genital tract. Granulomas, including sperm granulomas, were found in the epididymis of one bull.

Modern techniques for processing and distributing semen from such bulls create a situation, wherein thousands of cows may become infected with brucellosis. Semen plasma agglutination tests, seroagglutination tests, and bacteriologic examination of semen offer the best means of detecting an early or clinically inapparent infection.

Recent research has been directed toward differentiation of Brucella agglutinins in bovine serums on the basis of their heat stability at 65 C for 15 minutes. By this criterion a heat labile and a heat stable seroagglutinin have been demonstrated. Ultracentrifugation revealed that the heat labile seroagglutinin had a higher molecular weight than the heat stable seroagglutinin. The heat labile agglutinin was found in the serums of cattle known to be brucellosis-free; in the serums of calves after vaccination with Brucella abortus Strain 19; and in serums of heifers with persistent post-vaccinal seroagglutinin titers. It was also found in the serums of cattle recently exposed to virulent Brucella abortus and in serums of cattle that became infected. The heat stable agglutinin was found only in the serums of cattle that were infected or exposed to Brucella abortus. The heat labile



seroagglutinins were predominant at the onset of the disease, whereas the heat stable seroagglutinins became predominant as infection progressed. Heat stable *Brucella* seroagglutinins were an indication of exposure to *Brucella abortus*, whereas the significance of the heat labile seroagglutinins was not as readily apparent.

Acidified Plate test antigens at pH 4.0, 3.6, 3.4, 3.2, and 3.0 were evaluated in supplementary tests to clarify the status of "suspect" cattle to standard seroagglutination tube and plate tests for bovine brucellosis.

The serologic response of 57 vaccinated and 22 nonvaccinated animals was studied after conjunctival exposure in midgestation to about  $7 \times 10^7$  cells of virulent *Brucella abortus* Strain 2308. The brucellosis status of each animal at the termination of pregnancy was determined by bacteriologic examination of blood, milk, uterine contents and fetal organs.

The acidified plate antigens were compared for their inhibitory effect on serologic reactions of infected and noninfected cattle.

Inhibition of the suspect serologic reactions was directly dependent upon the magnitude of the original standard seroagglutination tube or plate test titer and the pH of the antigen. Serologic reactions in the lower suspect titer range were more readily inhibited. The inhibitory effect increased gradually as the final pH of the antigen-serum mixture decreased.

Since low level seroagglutination reactions involving specific *Brucella* agglutinins were frequently inhibited by the antigens, such antigens have little value in supplemental tests to determine the brucellosis status of "suspects" in an infected or problem herd. (NADL)

The University of Minnesota, under a cooperative agreement with the USDA, continued the studies of macroglobulins of milk and serum, but the emphasis was on developing methods of application of basic findings. Studies during the past year have been concerned with evaluating a new test procedure to detect macroglobulins for *Brucella* in cattle serums and milk, studying the occurrence and distribution of these agglutinins in a certified county, and studying their appearance and persistence in experimentally infected swine. (Minnesota)

The University of Wisconsin, under a cooperative agreement with the USDA, initiated a systematic study of the complement-fixation test for use as a diagnostic procedure in the research on brucellosis. A standardized method for the test has been developed. Preliminary data indicate that the test is a useful supplemental test for subjects in problem herds. It permits differentiation of infected vaccinated heifers from non-infected vaccinated heifers with persistent agglutinin titers. Other data comparing the CF with other supplemental tests are being analyzed.

In the immunochemical determinations, phenol extracts from Br. abortus have been compared with those previously obtained by some disruption of cells. These have been studied by serological and biological methods. (Wisconsin) (ADP al-3(Rev.))

## B. Vibriosis

The National Animal Disease Laboratory (NADL) workers reported their investigations were directed toward analysis of the cellular changes in the uterine endometrium due to V. fetus infection. Twelve virgin heifers were infected by natural service to a V. fetus-infected bull. At intervals of 8 to 122 days after initial exposure, the heifers were necropsied and uterine sections obtained for histopathological studies. Five unbred heifers and 7 heifers bred to a noninfected bull, were necropsied at intervals of 0 to 39 days after estrus or breeding and uterine sections were obtained for comparative studies as controls.

All heifers bred to the infected bull became infected at first service and microscopic studies of uterine sections revealed neutrophilic and lymphocytic periglandular infiltration of the endometrium that was not observed in non-infected heifers. The presence of a moderate inflammatory process suggested the underlying cause for lack of conception and subsequent pregnancy. Histologic differentiation was not observed in the cervicovaginal areas, cervixes, or ovaries of any heifers. Only 2 of 12 infected heifers had evidence of pregnancy when euthanized compared with 6 pregnant of the 7 non-infected heifers. (NADL)

The New York State Veterinary College, Ithaca, under a cooperative agreement with the USDA, continued research investigations on diagnostic procedures for vibriosis. One of the principal difficulties encountered in the control of vibriosis is the diagnosis of the asymptomatic carrier state in the bull. Cultural methods are not reliable enough for routine diagnosis in all bulls. The inoculation of test heifers, with material taken from suspect bulls, is a very expensive and time-consuming procedure. The fluorescent antibody technique provides a promising method which would be much more rapid and much less expensive.

Fluorescein conjugated rabbit gamma globulin against Vibrio fetus has been prepared. It has been tested on pure cultures of V. fetus and found to stain these organisms satisfactorily. Hyperimmune gamma globulin has been prepared against other species of vibrio and common contaminants. This will be used to insure specificity.

In the study of the incidence of vibrio fetus carrier bulls, it was found that both age and tenure were found to have a highly significant effect on the rate of infection. The frequency of infection was at a peak during the age range of 6 to 11 years, the most useful life span of AB proven bulls. The incidence of carrier status in bulls that entered the stud as young sires, increased almost five-fold as they entered their 7 to 8-year tenure period. The chance of exposure and infection increases with age and tenure.



Toxicity trials were conducted using nitrofurantoin drugs for the treatment of infected bulls. Furazolidone cream (1%) and Furacin solution (0.2%), have been tested in bulls at two and three times the usual number of treatment applications in an attempt to determine possible toxic effects. Six bulls (2 controls, 2 given 6 treatments, and 2 given 9 treatments) were used in this trial. Weekly semen samples were collected for 3 weeks prior to the start of the treatments, and were continued on all bulls until 10 weeks after the end of the treatments. No deleterious effects on the penile mucosa, semen quality or health of the bulls were observed. Semen volume, motility, concentration, and morphology were determined on all samples collected. Semen from all bulls remained normal throughout the experiment. (New York) (ADP al-9(Rev.))

### C. Tuberculosis

Research was continued at the Michigan State University under two contracts with the USDA. Reports submitted are as follows:

(Contract No. 12-14-100-6852(45)). During this year 27 calves which did not react to avian or mammalian tuberculin or Johnin were purchased. The herds of origin of these animals were reported as free of tuberculosis by the U.S. Department of Agriculture. These animals were divided into 9 lots of 3 each. Three lots were infected with a Group III mycobacterium which induced the greatest sensitivity in guinea pigs to mammalian tuberculin: 3 lots with a Group III mycobacterium which induced greatest sensitivity in guinea pigs to avian tuberculin, and 3 lots with a Group III mycobacterium which induced greatest sensitivity in guinea pigs to Edward's purified protein derivative-Batley (PPD-B). Three animals in each of 3 lots were inoculated via the intradermal route; 3 via intrauterine route, and 3 with an aerosol of the organism. Blood samples and tuberculin sensitivity data have been, and are being obtained at appropriate intervals. One animal from each lot has been or will be sacrificed at 2, 4, and 6 months post-inoculation, or earlier, if the animal's condition necessitates. Tissues from each animal were, or will be examined bacteriologically and pathologically to determine the infectivity of the organisms.

Serums collected are being examined for polysaccharide and phosphatide specific antigen. The precipitinogenic relationship of mycobacteria of human and animal origin has been investigated using antiserums produced in rabbits. Selected strains (24) of mycobacteria of human and animal origin have been examined for specific lipids by chromatologic fractionation and infra-red spectrophotometric analysis.

Studies on the change in virulence in selected atypical isolants induced by repeated passage through guinea pigs are in progress.

(Contract No. 12-14-100-5786(45)). The first work undertaken on this contract was the testing of samples of feed supplements of animal origin for the presence of acid-fast organisms. To date 107 samples have been examined.



Sixty animals to be used in the study were obtained from a herd in which no tuberculin reactors were found. In addition, the animals purchased had no detectible response to avian or mammalian tuberculins or Johnin. When tested with these products in the cervical region, the animals were transported to and are maintained in the barn used for the study in such a way as to prevent their contamination by other animals.

The work has progressed according to the schedule presented in the first semi-annual progress report of this project. More specifically, the animals were first tested for the presence of internal parasites, leptospirosis, and brucellosis. Then they were tuberculin tested, using mammalian tuberculin in the caudal fold. On February 13, the feeding of the experimental ration was started. Group A (15 animals) were fed the control ration; Group B (15 animals) the control ration with killed Mycobacterium bovis added; Group C (15 animals) the control ration with killed Mycobacterium avium added, and Group D the ration containing meat and bone scrap and steamed bone meal as detailed in the previous report.

Caudal fold tuberculin tests using mammalian tuberculin were performed on different lots of 5 animals of each group at 20, 30, and 40 days following the start of feeding the experimental rations. All animals were tested with mammalian tuberculin injected into the caudal fold after being fed the experimental rations for 100 days. (Michigan) (ADP al-13(Rev.))

#### D. Mucosal-Respiratory Disease-Complex

Research studies were continued at the National Animal Disease Laboratory. A soluble antigen present in infectious tissue culture fluids was separated from the infective virus particle by ultracentrifugation of two serologically related strains of bovine viral diarrhea viruses, NADL-MD and Oregon C24V.

Neutralizing antibodies against the two viruses were absent in four hog cholera antisera, but present in significant titer in a commercially prepared antiserum. Precipitin tests utilizing the agar double diffusion technique formed a single line of identity between the concentrated soluble antigen of both viruses and NADL-MD and hog cholera antisera. No lines were observed using concentrated virus pellet, noninfected embryonic bovine kidney cell antigens, specific pathogen-free calf serum or swine sera.

The relationship between the antigens of bovine viral diarrhea and hog cholera were investigated cooperatively with the Hog Cholera Project. Specific staining of antigen within bovine embryo kidney tissue culture cells, infected with either Oregon C24V or NADL-MD bovine viral diarrhea virus, was accomplished using fluorescein-conjugated swine anti-hog cholera or bovine anti-viral diarrhea globulin. Also specific staining of antigen within pig kidney tissue culture cells, infected with hog cholera virus, was accomplished using the same two types of conjugates. Specificity was confirmed by appropriate controls.



It was found that immunofluorescence was a convenient and sensitive method for determining an antigenic relationship between hog cholera and bovine viral diarrhea viruses. (NADL)

Colorado State University, Fort Collins, under a cooperative agreement with the USDA, made investigations which were reported as follows: Studies were conducted on the longevity of immunity to infectious bovine rhinotracheitis (IBR), presently considered in the Mucosal-Respiratory Disease-Complex. During the past year, the serum neutralization titers of cattle which are kept in the isolation units did not show lowering of titer. There was no difference in serum titers between the group which was infected intratracheally and the group which was infected intramuscularly.

In studies on the susceptibility of mule deer to IBR, 18 of 50 deer, obtained from different areas in Colorado, were found to have significant antibody titer of IBR, 12 of them had equivocal titers and 20 of the deer were free of IBR titer. Twelve of the 20 deer were used for testing the susceptibility of IBR. These deer with significant titer of IBR were also challenged with IBR virus to see whether it would produce any clinical reactions. The results were negative. In addition to the deer, 3 elk and 1 antelope were also inoculated with IBR virus, but they did not show any clinical reactions.

Two to 4 days after injection of IBR virus intratracheally, clinical reactions were shown among the deer. Generally they showed anorexia, depression, excess salivation and respiratory distress such as increased respiration rate, dyspnea and occasionally dry cough. Two of the deer also showed excess serous nasal discharge. One deer showed conjuncto-keratitis 4 weeks after infection at which time the animal was normal otherwise. All the clinical signs were milder than those of cattle. Hematological values of white blood cells and differential counts were within the normal range.

Virus was isolated from the nasal secretions for 7 days after inoculation of virus. Virus was also isolated from the deer with conjuncto-keratitis from the eye swab. One virus isolation was obtained from the fecal swabs from another deer. This virus isolated from the fecal swabs was obtained from a deer also 4 weeks after injection of virus while there was no sign of sickness which could be observed. No specific clinical reactions were observed after challenge, which was 5 weeks after initial infection. There was no death loss resulting from IBR infection.

The pattern of serological response was similar to that of cattle. The measurable antibody titer appears 5-7 days after infection. It took 10-12 days to reach the height of the antibody level of  $10^{4.5}$ . The results obtained show evidence that deer are susceptible to and play a role in the epizootic of IBR.

In a study of the pathology of IBR in relation to abortion, five groups of pregnant cows, with 5 head for each group, were used for pathological and virological studies. Ten additional cattle were used as controls.

Group	No. of cattle	Stage of pregnancy	Time between infection and fetal material collected
I	10	1st-3rd trimester	---
II	5	1st trimester	3½-5 weeks*
III	5	1st trimester	4 weeks
IV	5	3rd trimester	2 weeks
V	5	3rd trimester	3-4 weeks*
VI	5	1st trimester	1 week

\*Two abortions occurred after infection and 1 dead fetus found in uterus.

The abortion occurred in both first and third trimester pregnancy. The approximate time for producing abortion after infection with IBR virus in pregnant cows is approximately 3-5 weeks. Due to the spontaneous abortions in Group II and V, the rest of the pregnant cows in those groups were sacrificed, so that the specific pathological changes could be traced. During this process, one dead fetus was found from each group which gave more convincing evidence that the fetal materials were obtained close to the abortion. Materials collected are being prepared for pathological study and virus isolation. (Colorado)

Purdue University, Lafayette, Indiana, under a cooperative agreement with the USDA, reported sporadic cases of acute virus diarrhea and mucosal disease continue to occur in Indiana. Serums obtained from such herds contain high titers of neutralizing antibody against the C24v strain of virus.

Typical cases of experimental virus diarrhea followed the inoculation of susceptible calves with tissues from two of three field cases of suspected virus diarrhea. In addition to demonstrating the current presence of active virus in the herds of origin these trials provided two new isolates of virus diarrhea agent which will be tested for immunologic relationships with known strains and possible cytopathogenicity.

An agent which is cytopathogenic for ovine thyroid cell cultures was propagated in these cells during the serial passage of VD 46 virus. Subsequent study suggests that the cytopathogenic agent is not VD 46 virus or a bacterium. The identity of this (viral?) agent has not been established.



A metabolic inhibition test for the assay of polio virus was adapted to the assaying of VD-MD viruses. Virus effects were noted only when undiluted and  $10^{-1}$  dilutions of virus were employed and so the sensitivity of the assay under the conditions employed was judged to be impracticable.

The development of a passive hemagglutination test for the detection of antibodies against VD-MD was attempted for its obvious advantage as a diagnostic test and as an aid to laboratory study of this disease complex (cross protection tests in animals and neutralization tests in tissue culture are definitive but costly and time-consuming). The test system developed employs C24v virus grown in bovine embryonic kidney cell cultures and tanned and labelled sheep red blood cells. Thus far only low titer agglutination reactions have been observed with selected field sera and with experimental sera with cells labelled with concentrated soluble antigens. However, these low titer reactions were observed only in post-inoculation samples, so studies are in progress utilizing other strains of VD-MD virus and a wider range of experimental and field case serums.

Paranatal hematologic values for Caesarean-derived calves are reported. The major developmental changes appeared to be complete by weeks 8 to 10. The lack of immature neutrophils at weeks 8, 10, and 12 indicated a relative freedom from bacterial infections. Total serum proteins increased throughout the first 12 weeks with marked changes in the relative percentages of the globulin components.

Birth weight, 180-day weaning and yearling weights with average daily gains are presented for the SPF cattle herd. This limited data indicates that the major differences in this regard, between Caesarian-derived and second generation calves, occurs prior to weaning.

A bacteriological survey directed to the detection of selected bacterial pathogens was conducted in the SPF cattle herds. Forty animals were evaluated in March, 38 in April, and 32 in June. Three specimens were taken from each animal each month - nasal swabs, eye swabs, and fecal specimens. Large numbers of several species of bacteria were isolated but during the period of this survey none of the animals appeared to be harboring bacteria known to be capable of inducing disease in cattle. This appeared to be true even though herds on surrounding farms continued to present a history of diseases associated with the specific bacteria sought in this survey. (Indiana)

Iowa State University, Ames, under a cooperative agreement with the USDA, reported work on a new serological strain of virus diarrhea virus.

Identification: The new strain of virus diarrhea virus referred to as MDI-2 was a distinct serotype of virus diarrhea. The MDI-2 strain, however, does share a common soluble antigen with other virus diarrhea strains. This common antigen can be detected by the fluorescent antibody technique.



It is evident that the new strain is serologically distinct because of three reasons - 1) reciprocal cross protection tests with MDI-2 and other virus diarrhea agents failed to demonstrate cross protection: 2) specific rabbit prepared antisera to the MDI-2 agent and to C-24-V virus prototype failed to show reciprocal cross neutralization, and 3) vaccination with MDI-2 failed to protect pigs from a subsequent challenge with hog cholera virus.

Characterization: Production of Disease in Calves: MDI-2 produces a very distinct experimental disease in calves inoculated intravenously. The reaction is characterized by a diphasic temperature response with a concomitant leucopenia associated with the primary temperature elevation. Clinical signs of anorexia, dyspnea, diarrhea, increased lachrymation, and salivation were present.

Properties of the Virus:

1. Structure and classification: Electron microscopic studies revealed that the virus has a structure and a developmental cycle identical to that of myxoviruses in general. Since our antigenic studies revealed that the MDI-2 agent is related to other strains, we now conclude that the entire group of virus diarrhea agents are myxoviruses.

2. Ether sensitivity: The MDI-2 agent is ether sensitive which would support the fact that it is a myxovirus since all members of this group are ether sensitive.

3. Antigenic characteristics: We have demonstrated by a combination of serum neutralization trials, fluorescent antibody tests, and double diffusion studies in agar, that two viral antigens exist. V soluble antigen appears to be responsible for cross fluorescence studies with other agents which are not related insofar as they do not cross neutralize. The soluble antigen will react with anti C-24-V antiserum in a double diffusion test.

4. Growth characteristics: MDI-2 virus adsorbs to testicular cell monolayers rather slowly. A 3-hour adsorption time was necessary to insure 98 percent adsorption. By fluorescent antibody studies, we have determined that the first antigens appear within the nucleus at 3 hours. By electron-microscopy, these antigens may be identified as the nucleocapsid which most probably contains ribonucleic acid and protein. The fluorescence gradually disappears from the cell nucleus and appears in the cytoplasm, although there is a period where both nuclear and cytoplasmic fluorescence is seen. The intense fluorescence observed at 10 hours is correlated with densely packed complete virus particles adherent to the cell surface as observed through the electron microscope.

Virus penetration is passive in that the virus particles are apparently taken in by the normal pinocytotic process. The viral envelope is intimately attached to the plasma membrane lining the vesicle and is apparently broken



down within the vesicle. All of these events could be observed in electron-photomicrographs.

It is obvious both from fluorescent antibody studies and electron microscopy that the virus is held at the surface of the cell prior to release which is typical of myxoviruses in general.

This work has presented some very important considerations both from an applied aspect and from a very basic concept. Since this agent has been shown to be a new serological strain of virus diarrhea, it follows that the existing vaccines which only incorporate one virus would not fully protect animals against virus diarrhea.

From a basic aspect it was shown that the MDI-2 agent is related to other virus diarrhea strains by a common soluble antigen. We have also shown that this cross reaction does not exist with the noncytopathogenic virus diarrhea strains. This brings up the interesting point as to the mechanism of cell-killing by the virus. It may be that the presence of the soluble antigen actually kills the cells and that the absence of it is correlated with the noncytopathogenic strains. This idea does have a practical side because of the fact that MDI-2 strain actually is more virulent than the noncytopathic Sanders Agent.

Viral Isolation Attempts from Cattle: It was shown experimentally that the MDI-2 agent produces signs referable to both the digestive tract and the upper respiratory tract. This agent was reisolated from a nasal swab taken from an experimentally infected animal. Attempts to isolate agents from herds clinically affected with mucosal disease were negative. These included isolation attempts from 78 animals. Efforts to isolate agents from three "normal" herds were successful. The agents are being studied.

Pathology of experimental virus diarrhea: A comparative study of the pathology produced by 6 mucosal disease-viral diarrhea agents in calves under experimental conditions has been described. Four of the agents produced similar lesions in the digestive mucosa and lymph tissues which corresponded to the early lesions reported for field cases of mucosal disease-viral diarrhea. Two of the agents, which later were identified as strains of infectious bovine rhinotracheitis virus, produced similar but not as severe lesions as the other 4 agents. In addition, these agents produced multiple foci of necrosis in the adrenal cortex.

Considering the similarity of the clinical syndrome, clinical pathology, immunological protection and lesions produced, it would appear that 4 of the agents studied are closely related. The other 2 agents also appear to be related to each other but not to the other four. (Iowa) (ADP al-14(c)(Rev.)

## E. Mastitis

The work conducted at the National Animal Disease Laboratory, Ames, Iowa, was reported as follows: Twenty-five dairy cows with udders free of hemolytic, coagulase-positive staphylococcic infections were tested for blood serum alpha and beta antitoxins for periods up to 2 years. The number of serums positive for these antitoxins and the average antitoxin titers of the positive serums increased with the age of the animals and reached maximum levels during the second lactation period. However, a progressive increase in antitoxin titers during the test period was not apparent when animals were considered individually. In most animals, the titers developed to certain levels and remained relatively stable or declined to levels below 1 international unit (I.U.) of antitoxin.

Three laboratory strains of Streptococcus pyogenes were cultured by daily transfer in a medium composed of 22 amino acids, 2 purines, 1 pyrimidine, B vitamins, inorganic salts and buffered with 0.1 M phosphate buffer, pH 7.0. The basic medium is that described by Williams, Cornell Exp. Sta. Bull. No. 337 (1955). It was modified by the addition of L-glutamine, ammonium acetate and 0.1 M phosphate. The extra buffer increased growth more than 2-fold. Daily transfers of a 1 percent inoculum resulted in good growth. Optical densities measured in 24 hours with a Klett-Summerson colorimeter and 550  $m\mu$  filter were 0.40-0.50. One percent glucose was completely fermented to quantitative yields of lactic acid. (NADL)

At the University of California, Davis, under a cooperative agreement with the USDA, a strain of bacteria called Aerobacter aerogenes has been employed which may occur in the environment of dairy cattle. It was selected because it can be readily identified and at the same time will serve as a representative of the fecal (manure) bacteria. Such bacteria, called coliform organisms, are not commonly disease-producers but under certain circumstances of commercial dairying, may enter the mammary gland and produce a very severe inflammatory disease which may lead to death of the cow.

Investigations at California have shown that severe disease results only when a large bacterial population has been produced within the mammary gland. The effects on the cow are not directly related to bacterial growth but rather to destruction of the organisms by the defenses of the body. During multiplication of the organism in the mammary gland, there are no outward signs of the disease, but in time, a reaction sets in (inflammatory) which immediately destroys the organism in large numbers thereby releasing a poisonous substance in high concentration (endotoxin); it is this material released from the bacteria which produces peracute mastitis and which may even lead to death of the cow.

The production of a mild irritation of the mammary gland prior to exposure to the coliform bacteria prevented growth of the organism and the development of severe mastitis. The most important factor in a response to irritation



to mammary tissue is an infiltration of cells (leukocytes) from the blood. It was shown that cells infiltrating into the milk at levels generally accepted as representative of high normal values (250,000 to 500,000 per cc) were capable of inhibiting growth of coliform organisms within the udder and therefore were highly protective against coliform mastitis. The practical implications are that as cows become older and their mammary tissues respond to oft-repeated irritation inherent in the milking process, the leukocyte activity so engendered also at the same time protects the cows against coliform organisms. Therefore, extensive use of antibiotics to reduce mild inflammatory reactions would appear to be inadvisable. (California) (ADP al-15(R))

#### F. Epizootic Bovine Abortion

The University of California, Davis, under a cooperative agreement with the USDA, reported that during the past year the following findings were made in studies of epizootic bovine abortion (EBA): 1) ingestion, as hitherto believed, does not appear to be the manner in which the virus gains entrance to the body under field conditions: 2) cattle do not become refractory to abortion following exposure, as virgin heifers, to virulent virus: 3) inactivated EBA virus vaccines do not protect cattle against abortion, and 4) the EBA virus appears to be identical with, or closely related to, the virus of enzootic abortion of ewes (EAE).

These findings suggest that the EBA virus is venereally transmitted and an infection immunity develops following exposure. However, it does not develop rapidly enough to prevent abortion in the initial pregnancy. Thereafter, cattle are refractory to reinfection and abortion with this virus because the infection immunity becomes fully operative after termination of the initial pregnancy. (California) (ADP al-21)

#### G. Paratuberculosis (Johne's Disease)

Research workers at the National Animal Disease Laboratory, Ames, reported that studies were conducted to devise an improved technique for primary isolation of Mycobacterium paratuberculosis. Trypsin digestion of infected intestinal mucosa, followed by decontamination with 1N NaOH, was effective in preparing the inoculum for primary cultivation. A lymph node-egg yolk medium was superior to several other mediums for primary cultivation and subcultivation of newly isolated strains.

A herd of cattle, ranging in size from 161 to 195 head, in which Johne's disease was known to exist, was tested periodically with intradermic johnin. Selected tissues of all animals removed from the herd were examined after slaughter for Mycobacterium paratuberculosis. The following observations were made on 96 animals eliminated from the herd during this 5-year study:

Forty-six cattle reacted to intradermic johnin. Fifteen of the reactors developed clinical evidence of Johne's disease, and 21, including these 15, were found to be harboring M. paratuberculosis after slaughter.

Twenty of 50 nonreactors were also found to be harboring the bacillus after slaughter, and 10 of these had developed clinical evidence of Johne's disease; a total of 20 cattle, including these 10, were found to be harboring M. paratuberculosis.

Twenty-six cattle that reacted to intradermic johnin were tested periodically for several years, and the following observations were made: a) sensitivity persisted for only 6 months in 12 cattle, 3 of which were found to be infected with M. paratuberculosis when examined after slaughter: b) Sensitivity persisted for 12 months in 5 others, 1 of which was found to be infected when examined after slaughter: c) sensitivity persisted for 36 months in 5 cattle, 3 of which developed clinical evidence of Johne's disease: d) sensitivity was intermittent with no particular pattern in 4 cattle. One of these animals developed clinical evidence of Johne's disease, but in the others there was no evidence of the disease when examined after slaughter. (NADL) (ADP al-35)



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**FOOT-AND-MOUTH AND OTHER EXOTIC INFECTIOUS DISEASES OF CATTLE**  
**Animal Disease and Parasite Research Division, ARS**

Problem. Responsibility for protection of the Nation's livestock industry against diseases, including those of foreign origin, was delegated to the USDA in 1884. Thereafter, contagious bovine pleuropneumonia eventually was eradicated from the United States, thus reopening European markets for American cattle. Ever since then the Department has successively imposed practicable, scientifically justified barriers against introduction of such dangerous exotic diseases as foot-and-mouth disease and rinderpest. The Plum Island Animal Disease Laboratory was established for scientific support of measures for protection against these and other foreign diseases of animals, following the direct threats of spread of foot-and-mouth disease from Mexico and Canada (1946-1954). Foot-and-mouth disease, which is capable of reducing overall productivity by 25 percent in areas where it becomes established, persists in most major livestock-producing countries, except Central and North America, Australia, and New Zealand. Rinderpest continues to be a serious disease problem in Africa and Asia; it is capable of killing 90 percent or more of the cattle that are exposed to it. Other diseases, such as contagious bovine pleuropneumonia, Rift Valley fever and East Coast fever continue to exact severe tolls in other parts of the world. Possibilities of entry of these diseases into the United States continue, despite all precautions, primarily because of the progressively increasing scope, speed and extent of modern international transportation. The purposes of the Plum Island laboratory are development of basic information applicable to protection of the Nation's livestock from foreign animal diseases; development and maintenance of competence in diagnosis of these diseases, and fundamental research on the biological, chemical and physical properties of the infectious agents that may be useful in prevention, control and eradication of these diseases.

**USDA PROGRAM**

The Department has a continuing long-term program involving veterinarians, biochemists, biophysicists, microbiologists, and pathologists, engaged in basic and applied research in this problem area. All of this research is being conducted on the following diseases at the Plum Island Animal Disease Laboratory, Greenport, Long Island, New York, except for supplemental field studies on vaccines in The Netherlands.

The Federal scientific effort devoted to research in this area, conducted solely at the Plum Island Animal Disease Laboratory, totals 25.0 professional man-years. This effort is divided among sub-headings as follows:

Pathology -- foot-and-mouth and other exotic diseases 1.0

Fluorescent antibody technique to locate viruses 1.0

Studies of foot-and-mouth disease vaccine 4.0

Immunological investigations to determine the mechanism of antibody formation using viruses of exotic animal diseases 0.5

Immune response to types and sub-types of foot-and-mouth disease virus 1.5

Quantity production of foot-and-mouth disease virus 2.0

Microcinematography of infected cells 0.5

Establishment and characterization of cell lines and cell strains 1.5

Interaction between foot-and-mouth disease virus and host cells 1.0

Genetic biochemistry of foot-and-mouth disease and other exotic viruses 1.0

Effects of natural and artificial stresses on foot-and-mouth disease virus 1.0

Bulk freeze-drying of foot-and-mouth disease virus vaccines and anti-serums 1.0

Rinderpest of cattle 2.5

Survival and transmission of foot-and-mouth disease virus in semen 1.5

Identification, purification, characterization of foot-and-mouth disease virus 2.0

Immuno-chemical investigations of foot-and-mouth disease 1.0

Survival and inactivation of foot-and-mouth disease virus in meat and meat by-products 1.0

Biological mechanisms of natural resistance and susceptibility to foot-and-mouth disease virus 1.0

Work was continued under a PL 480 grant to the Biological Institute, Sao Paulo, Brazil, for a 5-year study of tissue culture of indigenous strains of foot-and-mouth disease virus, and experimental field vaccination.



## REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Pathology - foot-and-mouth and other exotic diseases.

The lingual pathology produced by the virus of foot-and-mouth disease was studied in 165 specimens using a technique designed to record and correlate gross, subgross, and microscopic characteristics of large numbers of lesions. The essential pathological alterations consisted of necrosis of epithelial cells in the stratum spinosum, intercellular edema and granulocytic infiltration. These changes caused development of circumscribed, slightly elevated blanched areas in the lingual mucosa, to which the designation "initial lesion" was applied. A considerable proportion of initial lesions developed promptly into full blown vesicles by separation of the diseased mucosa from the underlying tissue, and the fluid was probably lost through cracks in the stratum corneum. In such areas the desiccating, necrotic mucosa became discolored and the lesion had the gross appearance of a necrotizing rather than a vesicular process. This peculiarity of lesion development was attributed to firm attachment of the thick bovine lingual mucosa by the numerous, well-developed conical papillae.

Lesions in the interdigital skin were basically similar in their initial development but failure to vesiculate was exceptional. In this area, the necrotic edematous skin pulled away easily from the dermal papillae and large vesicles developed. The stress and motion to which the interdigital skin is subject was probably an important contributing factor. (ADP a8-1(R)).

B. Fluorescent Antibody Technique to Locate Viruses.

An indirect fluorescent antibody (FA) technique for foot-and-mouth (FMD) evaluation of cattle serums was studied. The reacting system included commercial fluorescein-conjugated rabbit anti-bovine globulin, calf kidney cell cultures infected with foot-and-mouth disease virus (FMDV), rhodamine bovine albumin and test cattle serum. Serums from 55 cattle were evaluated for FA reaction and the results were related to FMD virus experiences of the animals and to their serum neutralization indices.

Serums from cattle that had developed lesions of FMD consistently gave positive or (in two instances) suspicious FA reactions. The different types of FMDV could not be distinguished one from another by the FA reaction when serums of cattle infected with the 7 types were used. The FA reaction was detectable in serums from two steers as early as 6 days and in the serum of one steer as late as 2 years after inoculation with FMDV. One serum tested two years after inoculation gave a negative FA reaction.

Serums from cattle that had not developed lesions of FMD consistently gave negative FA reactions. This included cattle in the following groups: normal controls, convalescing from vesicular stomatitis and virus diarrhea, and animals immunized with an experimental vaccine. (ADP a8-2(R))



### C. Studies of Foot-and-Mouth Disease Vaccine.

The efficacy of swine, bovine, and baby hamster kidney cell cultures for the isolation, growth and assay of foot-and-mouth disease virus has been determined. These studies were performed with virus newly isolated from infected cattle. Swine kidney cells were preferable to bovine cells for virus growth and assay when low passage virus was used. Baby hamster kidney cells were very satisfactory and have the advantage that they can be produced in continuous culture. This cell line should prove to be a valuable asset in vaccine investigations.

Studies on the inactivation of foot-and-mouth disease virus indicate that formaldehyde does not reliably kill the virus. The more sensitive cattle tongue inoculation procedure for detecting possible residual live virus in formaldehyde treated preparations revealed that, while tissue culture and suckling mice tests gave no evidence of live virus, the cattle test readily demonstrated it. This more sensitive test thus indicated that formaldehyde was of questionable value for preparation of vaccines of assured safety. The compound, acetyl-ethylene-imine was used to prepare a lot of vaccine that did not infect cattle. This preparation is under potency evaluation. Other inactivating agents will also be examined to determine their possible use for producing vaccines.

Studies to establish the relative immunizing value of different vaccine preparations have been performed. Small animals, e.g., guinea pigs, are being used to circumvent the expense and difficulty of performing such tests in cattle. These studies revealed that the antibody produced by guinea pigs in response to the vaccine changed in its physical, chemical and serological characteristics with time following inoculation. As a result, interpretation of the results will be difficult until the significance of the two different types of antibodies produced is established.

Cooperative research on the extent of immunity of foot-and-mouth disease vaccine conducted in Amsterdam, Holland, revealed that knowledge of the extent and duration of immunity following vaccination against FMD is essential for the proper evaluation of vaccines and scheduling of field vaccinations. Because large numbers of animals are required over long periods of time, such studies can best be pursued in areas where the disease is enzootic and where field vaccination is routinely practiced. Studies in Holland in cooperation with The Netherlands Ministry of Agriculture have continued toward the evaluation of immunity of cattle vaccinated and held under field conditions. Twelve herds, consisting of approximately 400 cattle, are included in this study. Serum antibody levels against type O and A foot-and-mouth disease of animals vaccinated two or more times remain high over a 2-year period. Eighty per cent of those cattle which had received several annual field vaccinations and which were challenged 16-48 months later with virulent FMDV, showed resistance to the disease. In general there appears



to be a good correlation between serum antibody level and immunity, and studies will continue to further evaluate and define this relationship. (ADP a8-8(R))

#### D. Immune Response to Types and Sub-types of Foot-and-Mouth Disease Virus.

Cattle infected with foot-and-mouth disease continue to have virus neutralizing substances, i.e., antibodies, in their blood serum for at least five years after infection. Of three animals kept for this period following infection, one animal retained the ability to resist infection when re-exposed to the virus. Another group of cattle studied also demonstrated that antibody is present in sufficient amount for extended periods following infection. This was revealed by precipitation reactions performed by the agar gel method. It was also found that antibodies produced by cattle early in the course of an infection are different than those produced later.

It has been found that calves born of immunized dams do not have antibody to the virus in their serum; however, within 2 hours after receiving colostrum, antibody is found to be present. The transfer of antibody from dam to the calf's serum may be blocked by feeding skim milk or other proteins to the calf before it is allowed to receive colostrum from the vaccinated dam. It was not possible to immunize calves having moderate level of antibody circulating in their blood. It was necessary to let the colostrum-obtained antibody reach low level before satisfactory active immunization could be accomplished. This information is of importance in areas where foot-and-mouth disease occurs, as it is necessary to know at what age calves should be immunized for satisfactory protection. (ADP a8-11(R))

#### E. Quantity Production of Foot-and-Mouth Disease Virus.

A scheme for rapid modification of foot-and-mouth disease virus (FMDV) populations was developed by growth of the virus in progeny of tissue culture cells that survived infection. Plaque size diminished rapidly as virus was maintained in these cells, and, eventually, visible plaques were not produced. As modification of the virus population progressed, pathogenicity for mice and steers decreased with retention by the virus of significant levels of immunogenicity.

Some basic aspects of FMDV inactivation by glycidaldehyde (GDA) were investigated. The minimal concentration required to inactivate high concentrations of FMDV was between 0.008 and 0.12% GDA. The time required for inactivation of the virus was 105 minutes.

A major part of the effort devoted to this line project was spent in instructing and advising scientists in foreign countries on the growth of FMDV by tissue culture methods. Three months were spent in Turkey at the request of the United States Agency for International Development and the Government of Turkey. During this time a laboratory, consisting of 14 rooms was designed, established, and equipped with laboratory furniture, carts,

trucks, etc. More than 200 different laboratory items were ordered - mostly from the United States. Advice was given on organization of the laboratory and on production procedures.

One month was spent at the Razi Institute in Iran with personnel who requested advice on large-scale tissue culture media production.

Three weeks of instruction were provided at the Plum Island Animal Disease Laboratory to two scientists from Italy on production of FMDV by tissue culture methods. (ADP a8-12(R))

#### F. Microcinematography of Infected Cells.

Two black and white prints of each of the following films have been prepared:

Cytology I	-18676S	Cell Survival and Cell Culture Regeneration after Infection with Vesicular Stomatitis Virus - New Jersey Type.
Cytology II	-18675S	Cell Survival and Cell Culture Regeneration after Infection with FMDV-A119-BK8.
Cytology III	-18677S	Cell Survival and Cell Culture Regeneration after Infection with FMDV-A119-PB106.
Cytology IV	-18674S	Cytopathic Effect of Rinderpest Virus in Tissue Culture.

The following films (reversal film) have been prepared:

Cytology V	-18673S	Cytopathic Effect of Rinderpest Virus in Tissue Culture.
Cytology VI	-18672S	Cytopathic Effect of FMDV in Lamb Testicular Cells.

Titles will be redone and prints made of the last two films. (ADP a8-13(R))

#### G. Establishment and Characterization of Cell Lines and Cell Strains.

A lamb testis cell line developed at the Plum Island Animal Disease Laboratory, was used in microcinematographic study of cellular reactions to infection with foot-and-mouth disease virus (FMDV). This lamb testis cell line was also used in the development of fluorescent antibody technique to locate FMDV in the cell. (ADP a8-14(R))

#### H. Interaction Between Foot-and-Mouth Disease Virus and Host Cells.

Analytical ultracentrifugation was used to assess the efficacy of physical and chemical separations of 7S and 19S antibodies in sera of guinea pigs



and cattle convalescing from foot-and-mouth disease. A one-step preparative ultracentrifugal procedure separated the two antibody classes. The precipitin reaction was used to determine antibody stability in several solvents.

Foot-and-mouth disease virus was labeled with  $P^{32}O_4$  and leucine-3,4- $H^3$ . Three classes of RNA with sedimentation coefficients ( $s_{20,w}$ ) of about 4S, 12S and 23S have been isolated from bovine-kidney culture cells.

A stable baby hamster kidney cell line, obtained from the University of Glasgow, was grown in inexpensive media in roller bottles to populations of about 625 million cells per bottle in 6 days without fluid change. Infection with FMDV yielded fluids containing  $10^{8.5}$  to  $10^{8.8}$  PFU/ml. It appears feasible to scale the method upward to produce many milligrams of virus during a single week.

Suckling mice which survive one inoculation with high dilutions of FMDV which still contain appreciable numbers of physical virus particles die when reinoculated 3 to 4 days later with the same statistics as control animals. This suggests that not all FMDV physical particles are lethal and that only those mice die which receive a lethal particle amongst the many administered. (ADP a8-17(R))

#### I. Genetic Biochemistry of Foot-and-Mouth Disease and Other Exotic Viruses.

Heat denaturation of RNA obtained from virtually pure FMDV by phenol treatment was indicative of pure single-stranded RNA. Its temperature of half-melting ( $T_m$ ) in 0.02 M and 0.05 M sodium phosphate at pH 7.5 was 55° and 59°C, respectively. Such RNA contained guanine, adenine, cytosine and uracil in the molar fractions 0.24, 0.26, 0.28 and 0.22. RNA within FMDV did not heat denature until after its release from the protein coat. In 0.05 M sodium phosphate at pH 7.5 this commenced abruptly at 54°C. The  $T_m$  was 70°C. Redenatured virus melted very similarly to phenol-derived RNA with a  $T_m$  of 59°C.

Guanidine reversibly decreased FMDV production in bovine kidney cultures. This inhibition occurred during the latter stages of virus maturation and was not reversed by arginine or urea, both of which are structurally related to guanidine. Virus adsorption by cells was not affected. Para fluorophenylalanine inhibited virus reduplication in cells grown in serum-free medium, but not when serum was present. 2,6-diamino-3 phenylazopyridine hydrochloride (Pyridacil) reduced virus production possibly through its cellular toxicity. (ADP a8-18(R))

#### J. Effects of Natural and Artificial Stresses on Foot-and-Mouth Disease Virus.

Trichlorofluoroethane (TTE) and chloroform are each capable of reactivating virus from a neutral mixture of virus and specific antiserum. One part of

the chemical TTE was mixed with two parts of the virus-serum mixture. The material was then centrifuged and the aqueous layer recovered. Such treatment after eight extractions yielded the maximum virus in the aqueous layer. Butanol was found to inhibit the action of TTE or chloroform to extract the virus from a neutral mixture.

Two subcultures of type A, strain 119 FMDV adapted to tissue culture were compared for stability. After 90 passages, the two lines differed when exposed to 60 C; one was inactivated in 60 minutes and the other in 30 minutes. The two subcultures differed in response to drying on slides at 37C and 20% relative humidity; one survived over 120 days while the other was inactivated in 35 days. Both sublimes of virus reacted alike to ultraviolet irradiation and were neutralized by the same antiserum.

Under the conditions of testing, the presence of phenol red in the virus medium did not modify the inactivation time of the virus by ultra-violet light. However, centrifugation at 10,000 r.p.m. for 15 minutes and subsequent exposure of the supernatant to ultraviolet light showed the virus in such preparations to be inactive after 2-hours treatment. Specimens not centrifuged had three logs of virus remaining after 3 hours exposure to ultraviolet light. Twenty ml volumes of virus suspension in a petri dish were used and the fluid film was 0.5 cm thick at a distance of 8 inches below the germicidal light. The intensity was 7/u watts/sq.cm. Tongue tissue suspensions of virus were centrifuged and exposed to UV under the same conditions as the tissue culture virus with the result that 2.5 logs of viral activity remained after 105 minutes of exposure to UV. (ADP a8-19(R))

K. Bulk Freeze-Drying of Foot-and-Mouth Disease Virus, Vaccines, and Antiserums.

Type A, strain 119 foot-and-mouth disease virus, dried in tissue culture fluid in 250 ml amounts at either chamber temperature or with a 37C heat input to the drying chamber, did not process or store well at 4, 23, or 37C for 3, 2 or 1 months, respectively. This is in contrast to the same virus in 4 ml volumes in ampules which showed no loss in titer after 29 months of storage at 4C. (ADP a8-20(R))

L. Survival and Transmission of Foot-and-Mouth Disease Virus in Semen.

Sixteen grade Hereford bulls were infected with foot-and-mouth disease virus (FMDV) by tongue inoculation. The virus strains used represented six of seven known types of FMDV. At various times after inoculation, semen was obtained from bulls by electroejaculation for inoculation into steers and suckling mice to recover virus and determine titers, and for insemination of heifers.

Foot-and-mouth disease virus was found in semen of 2 bulls as early as 12 hours postinoculation which was prior to appearance of clinical signs of infection. Thereafter, virus was found in semen of the 16 bulls in 55 of



65 attempts, for as long as 10 days postinoculation. The virus titer in semen was usually higher than in urine and sometimes higher than in blood samples taken simultaneously.

Artificial insemination techniques were used to place semen from infected bulls in cervical canal and vagina of 19 heifers. Four of the 19 heifers developed FMD. It was concluded that semen of bulls could contain FMDV prior to appearance of clinical signs and lesions of infection and that FMD may be transmitted by artificial insemination. (ADP a8-24)

#### M. Identification, Purification, Characterization of Foot-and-Mouth Disease Virus.

Foot-and-mouth disease virus was produced in roller bottle bovine kidney cultures. About 3 liters of virus were harvested each week containing  $10^{8.8}$  plaque-forming units per ml. Chemical and ultracentrifugal concentration and purification yielded 2 mg virus of at least 94% purity with maximal infectivities of  $10^{11.0}$  PUF/ml., specific infectivities of  $10^{14.0}$  PFU/gm and physical particles by electron microscope counting of  $10^{13.6}$  virus particles/ml. The virus contained 32% ribonucleic acid (RNA) and 68% protein and had a 1% extinction coefficient at 259 mμ of about 76. Its specific refractive increment was about 0.16 ml/gm. Viral RNA had an extinction coefficient at 258 mμ of approximately 220 and a max<sup>258/min<sup>230</sup></sup> ratio of 2.1. The protein coat subunit of the virus obtained by heat, acid or urea treatments had a sedimentation coefficient ( $s_{20,w}$ ) of  $12.2 \pm 0.3S$ . There is considerable, but yet unequivocal electron microscope evidence that FMDV is a icosahedron with 42 subunits in its coat protein. (ADP a8-25)

#### N. Immuno-Chemical Investigations of Foot-and-Mouth Disease.

Antibodies present in the blood serum of animals immunized against, or infected with, foot-and-mouth disease virus are known to play a major role in their resistance to subsequent exposure to the virus. It has been shown that these antibodies may be of two different types, and they appear at different times following initial exposure to either the living or inactive form of the virus. These two types of antibodies have different physical and chemical characteristics. The early appearing antibodies are larger molecules (19S sedimentation rate) than the later developed ones (7S sedimentation rate). They also migrate more rapidly under the influence of an electrical field (B-globulin mobility) than do the later ones (γ-globulin mobility). Other differences in physical-chemical characteristics have also been found. In addition, differences in their serological activity have been found. The late appearing antibody is able to fix complement while the early antibody is not. The latter finding is important in that it imposes a limitation upon our diagnostic capabilities. Knowledge of the physical, chemical, and immunological nature of these antibodies is vital for an understanding of how animals resist infections.



Progress on the immunological characterization of the foot-and-mouth disease virus and antibodies produced by animals in response to the virus is dependent upon the development of precise measuring techniques. Quantitative immunological measuring methods have been applied to purified and crude virus preparations successfully. These procedures have certain advantages over many regular physical and chemical methods in that only minute amounts are required for the tests, the tests are usually easier to perform, and they may often be done on samples containing contaminating substances that interfere with other assay procedures. Further refinement and application of these procedures will increase our knowledge of the structure of foot-and-mouth disease virus. (ADP a8-26)

O. Survival and Inactivation of Foot-and-Mouth Disease Virus in Meat and Meat By-Products.

It has been established that the primary sites where foot-and-mouth disease virus may survive in carcass and in boned meat from infected animals are lymph nodes, hemal nodes, blood clots and bone marrow or bone fragments. A new and previously unreported site for virus survival is the joint fluids. Virus was found in joint fluids as early as 12 hours and for as long as 5 days after inoculation of cattle. The virus survived in joint fluids throughout usual treatments given carcass meat. During boning operation, virus in joint fluids could contaminate butcher's knives and be spread over surfaces of cut meat. The protection afforded the virus by the joint fluid would permit virus survival under some adverse conditions on surface of meat chunks. Thus, virus in joint fluids could be a hazard in imported meat. (ADP a8-28)

P. Biological Mechanisms of Natural Resistance and Susceptibility to Foot-and-Mouth Disease Virus.

Mice vary in susceptibility to infection with foot-and-mouth disease virus. Factors which affect the susceptibility or which might be related to the variation in response have been investigated. (1) Suspensions of kidney cells from 1- and 2-week old mice produced FMDV earlier and to higher titers than cells from older mice. While 1- and 2-week old mice are in the most susceptible age range, this difference in virus multiplication might be related to factors other than the susceptibility of cell donors. (2) Mother mice are most susceptible to FMDV during the first two weeks post partum and then gradually become resistant. To determine if this development of resistance was associated with weaning of their young and a consequent decrease in milk production, a group of mothers was given new litters of 5- to 7-day old mice at weekly intervals to the 4th week post partum. Challenge with FMDV demonstrated that such mice were slightly more susceptible than similar mothers with original litters but much less susceptible than 6-day post partum mothers. (3) Pregnant as well as mother mice are susceptible to FMDV. Experiments demonstrated that mother mice maintained the high degree of sensitivity to bovine serum which they developed before mating but became less sensitive after delivery of their young. (ADP a8-29)



Q. Studies on Foot-and-Mouth Disease Virus.

A PL 480 Grant was made to Instituto Biologico, Sao Paulo, Brazil, to conduct studies on foot-and-mouth disease virus. A laboratory has been established for production of tissue cultures to use as a media for propagating foot-and-mouth disease virus, and in the routine isolation of the virus from specimens received from the field.

A swine kidney cell line has been propagated for more than 18 months. The cells have remained fully sensitive to at least 3 types of FMDV encountered in Brazil. Work on attenuation of FMDV by tissue culture methods in this laboratory is continuing but no significant findings have thus far been reported. (S3-ADP-2)

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PARASITES AND PARASITIC DISEASES OF CATTLE  
Animal Disease and Parasite Research Division, ARS

Problem. The cost of parasitic diseases to the cattle industry of the United States is estimated to be in excess of \$400 million annually. Disorders caused by parasites are ubiquitous, generally insidious and often overlooked entirely. Diagnosis is difficult and successful treatments for many of these diseases are not available. Moreover, management practices to avoid spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling or eradicating parasitic diseases so as to provide for healthy cattle, insure adequate supplies of parasite-free beef for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a more prosperous agriculture and the national economy.

USDA PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, parasitologists, pathologists and veterinarians engaged in both basic and applied studies directed to the development of measures for the solution to the high and extremely costly incidence of parasitism in cattle. Research is being conducted on parasitic diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 21.5 professional man-years. This effort is divided among subheadings as follows:

Ecological Factors Influencing Nematode Development 1.0 at the Animal Disease and Parasite Research Division, Regional Animal Disease Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Effects of Pasture Mixtures and Pasture Management on Control of Internal Parasites 1.5 at the Regional Animal Disease Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Acquisition and Effects of Roundworm Parasites of Cattle, as Influenced by Diet 1.5 at the Animal Disease and Parasite Research Division, Beltsville Parasitological Laboratory, Beltsville, Maryland.

Artificial Propagation of Protozoan Parasites 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Host-Parasite Relationships of Coccidia 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.



Ecology and Immunology of the Cattle Lungworm 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Clinical and Physiological Aspects of Roundworm Parasitism in Cattle 2.0 at the University of California, Davis, under a cooperative agreement with the USDA.

Investigations of Trichomonad Parasites 1.0 at the Animal Disease and Parasite Research Division, Regional Animal Disease Laboratory, Logan, Utah, and under a cooperative agreement with the Utah Agricultural Experiment Station, Logan.

Host-Parasite Relationship of Intestinal Worms Cooperia spp. 2.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Factors Influencing Internal Parasitism of Grazing Cattle 1.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Winter Coccidiosis (Bloody Scours) 1.0 at the Regional Animal Disease Laboratory, Logan, Utah, and under a cooperative agreement with the Montana Agricultural Experiment Station, Bozeman.

Anaplasmosis of Cattle 4.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland, and through a memorandum of understanding and other agreements in cooperation with State Experiment Stations in California, Illinois, Louisiana, Nevada, the State Veterinarian of Tennessee, the USDA Entomology Research Station, Kerrville, Texas, and The Delta Branch Experiment Station, Stoneville, Mississippi.

Investigations on Anaplasmosis, Piroplasmosis and Babesiellosis of Cattle, are under way through a PL 480 Grant, at the School of Veterinary Faculty, Montevideo, Uruguay.

The Interrelationship of Diet and Parasitic Infection in the Production of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

The Histochemistry of Gastro-Intestinal Nematodes of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Parasites of Cattle - Stephanofilarial Species 1.0 at the Animal Disease and Parasite Research Division, Regional Animal Disease Laboratory, University Park, New Mexico.

## REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Ecological Factors Influencing Gastro-intestinal Nematodes.

Investigations made at Experiment, Georgia, under the auspices of the Animal Disease and Parasite Research Division's (ADP) Regional Animal Disease Laboratory at Auburn, Alabama, showed a significantly greater number of larvae of T. axei was recovered from cultures made with feces when the host calf was on a hay diet than when it ate a grain (corn) ration. Grain added to feces containing eggs of T. axei and T. colubriformis completely prevented the development of larvae in cultures when compared to cultures without the grain additive. Undigested grain (corn) washed from the feces passed by a calf on a grain ration also inhibited the larval development when added to feces from a calf infected with T. colubriformis. Experiments have shown that a grain ration (corn) appears to have an inhibitory action on the development in the feces of the larvae of two species of cattle nematodes (Trichostrongylus axei, T. colubriformis). (Georgia)

Experiments at the ADP Regional Animal Disease Laboratory, Auburn, Alabama, have demonstrated that rabbits are infected by ingesting the third-stage larvae of the nematodes, Trichostrongylus calcaratus, T. affinis and Obeliscoides cuniculi. Rabbits do not become infected by placing larvae on the intact skin if precautions are taken to prevent ingestion of such larvae by the host. Trichostrongylus affinis has been found in the cottontail (Sylvilagus floridanus) at Auburn, Alabama. Preliminary studies indicate that this nematode inhabits the cecum and appendix and has a prepatent period of 10-11 days with maximum egg production between 11 and 14 days post-infection. In guinea pigs immunized to the ruminant parasite, Trichostrongylus colubriformis, the immune response apparently was directed against the parasitic third- and fourth-stage worms. On the other hand, parasitic third-stage larvae failed to stimulate any immunity in guinea pigs, fourth-stage larvae elicited a measurable degree of immunity, and fourth- and fifth-stage worms, combined, stimulated an almost total immunity. (Auburn, Alabama) (ADP bl-6)

B. Artificial Propagation of Protozoan Parasites.

Work was continued at the ADP Beltsville Parasitological Laboratory (BPL) on the artificial propagation of protozoan parasites for the purpose of determining essential metabolites. Studies to develop methods of producing, under artificial conditions, the blackhead parasite in large numbers have been hampered by the unavoidable appearance of yeasts in the cultures. Amphotericin-B, an antifungal antibiotic, was found to control yeasts in culture without damage to the parasites, provided cream from cow's milk was incorporated in the culture medium. Without cream, the parasites grew poorly, if at all. Cholesterol compounds which the blackhead parasite can use for food were without effect when used in lieu of cream. (ADP bl-22)



### C. Ecology and Immunology of the Cattle Lungworm.

It was found at the Beltsville Parasitological Laboratory that double vaccination with irradiated larvae of the cattle lungworm can be effective in protecting calves from becoming appreciably infected with adults of this parasite subsequent to a massive exposure to vigorous infective larvae of the worm, such as occur on pastures. However, the procedure was not invariably effective under experimental conditions. Variability in its efficacy was not dependent on rate of irradiation of the vaccine within the limits that have been tested. Inherent individual variation among calves in capacity to develop resistance appears to be a significant determinant of the efficacy of vaccination.

Calves can become highly resistant or immune to the development of a mature cattle lungworm infection as a result of vaccination with infective larvae of a lungworm of sheep. The experiments on which this conclusion is based appear to be the first to show that one species of livestock can be immunized against one of its worm parasites by exposing it to infection with a worm that inhabits a different species. (ADP bl-24)

### D. Clinical and Physiological Aspects of Roundworms in Cattle.

The School of Veterinary Medicine, University of California, Davis, under a cooperative agreement with the USDA, reported research to show: Phenothiazine prepared with an anhydrous aluminum chloride catalyst was found to be superior to phenothiazine N.F. and equal to purified phenothiazine when evaluated in mice infected with Nematospiroides dubius: The addition of the other insoluble impurities of phenothiazine N.F. to purified phenothiazine did not reduce the anthelmintic action of the latter product, and that thiabendazole was found to be highly efficient as an anthelmintic in clinical cases of gastrointestinal parasitism of cattle when used at a level of 100 mg/kg body weight.

Work was also conducted on iron metabolism and hemopoiesis. The studies, utilizing  $\text{Fe}^{-59}$ , were conducted in order to evaluate the red blood cell survival times in lambs as determined by the method of Baker and Douglas and that of Pollycove and Mortimer. It was found that the latter method gives a longer mean survival (131 days) than the former method (81.0 days). Definitive studies in 2 lambs, using the method of Eadie and Brown, indicated mean red cell survivals of 141 and 143 days.

Body surface monitoring of spleen, liver, and bone marrow indicated that the spleen of sheep may act as a labile storage pool for iron utilized in hemoglobin synthesis.

Analysis of tissues for  $\text{Fe}^{-59}$  between 1.1 hr. and 7 days following intravenous injection of  $\text{Fe}^{-59}$  labeled plasma in lambs, revealed that 4 to 21% of the injected isotope was in the liver, 0.00 to 2.5% in the spleen, 0.00 to 16.5% in muscle, 0.10 to 2.1% in the kidneys, and the remainder was in plasma, bone marrow, and red cells. (California) (ADP bl-25)



### E. Investigations of Trichomonad Parasites.

Research on this project at the ADP Regional Animal Disease Laboratory, Logan, Utah, resulted in the following findings: Electrophoretic methods used for protein separation were not practical for immunoelectrophoresis because large amounts of antigen are required for the immunodiffusion phase of the procedure. The apparatus constructed for this method was therefore adapted to a micro-method. The supporting medium was changed from starch gel to ionagar which does not allow separation of as many fractions or as sharp delineation as starch, but the separation is sufficient for immunodiffusion. For general antigenic characterization and comparison of antigenic makeup of various strains of T. foetus, the micro-method should suffice. By this method rabbit anti-T. foetus serum was separated into the 5 fractions. The diffusion phase of the technique has not been combined with the electrophoretic phase, but has been done separately and produces precipitin lines. For positive identification of antigens that may characterize or differentiate strains, the starch gel method may be more effective.

Anti-serum against T. foetus may be produced in rabbits by intravenous injections of washed live organisms. However, some rabbits do not tolerate the infection. Intracardial injections did not prove satisfactory, neither did the intraperitoneal method with any of the 4 strains of T. foetus used. The effort to produce antiserum against T. foetus in calves gave negative results.

Antigens for use with the gel diffusion phase of the immunoelectrophoretic technique to date have been the complete type consisting essentially of concentrated ruptured organisms. Three methods were used for rupturing the organisms--1) lysis by hypotonic solutions, 2) alternate freezing and thawing, and 3) blender grinding. Acceptible antigens have been prepared from freeze-thawing. However, blender-ground organisms gave most consistent results.

Gel diffusion reactions were produced in ionagar gel of several strengths prepared with several buffers of varying pHs. Therefore, it is anticipated that the gel diffusion procedure may be combined with the microelectrophoretic technique. In trials to date, however, considerable cross reaction between the two strains used have occurred. T. foetus was isolated from 10 of 280 samples taken from bulls during the year. This included herds under observation following known infection. (Logan, Utah)

Research was continued at the Utah Agricultural Experiment Station, Logan, under a cooperative agreement with the USDA, to learn more about pentatrichomonads that were isolated from the rumen and cecum of calves in northern Utah in 1961. This trichomonad grew in Diamond's modified Plastringe's, and cecal-extract media. No difference in response to different media or in morphology was found in the trichomonads from the rumen



and cecum. There were 3, 4, or 5 unequal anterior flagella, one of which was independent, a relatively high, full-length undulating membrane, a prominent costa, a narrow axostyle with long protruding tip and no chromatic ring, a subspherical or round-oval nucleus, and an oval parabasal body with one or more central granules. Mean measurements of 100 Bouin's-fixed, protargol-stained, rumen specimens from 24-hour Diamond's cultures were as follows: length, 7.8 microns (range, 5.7-10.0); width, 6.1 (4.2-7.9); protruding tip of axostyle, 4.0; anterior flagella, 9.7, 8.8, 7.2, 6.0, and 4.2; trailing flagellum, 5.4; nucleus, 2.5 by 2.0; parabasal body, 1.5 by 0.9; height of undulating membrane, 1.7. This trichomonad is similar to Pentatrichomonas hominis and may be identical with this species. (Utah) (ADP bl-26)

#### F. Host-Parasite Relationship of Intestinal Worms Cooperia spp.

Reported research from the ADP Regional Animal Laboratory, Auburn, Alabama, showed that calves inoculated with 250,000 Cooperia pectinata infective larvae made an average weight gain of only 6 pounds in 6 weeks, while non-inoculated controls averaged a gain of 25.3 pounds. Clinical signs of parasitism -- anorexia and enteritis as indicated by passage of abnormally soft stools -- appeared during the third and fourth weeks of infection, and these were accompanied by decreased levels of serum proteins and blood sugars. The effects were not as severe as those produced in previous studies wherein calves were inoculated with 350,000 infective larvae. Calves inoculated with 350,000 Cooperia oncophora larvae developed a mild parasitism, characterized by a short period of enteritis and some retardation on rate of gain. However, this parasite is not as pathogenic as a closely related species, C. pectinata. The intestinal worm, Cooperia pectinata, develops in the intervillar spaces of the duodenal mucosa, causing disruption of the intestinal villi, and evoking a catarrhal exudate.

It has been concluded from experiments at Auburn that some factor, or factors, operating within individual host animals (calves and lambs) are sufficient to affect significantly the size of infective third-stage larvae (Cooperia oncophora) developed from eggs laid by the infecting parasites. Some of these factors may be individual differences in the utilization of the diet by the host, or differences in the microbiota of the feces. (Auburn, Alabama) (ADP bl-27)

#### G. Factors Influencing Internal Parasitism of Grazing Cattle.

The Beltsville Parasitological Laboratory (BPL) research workers reported that calves and older cattle infected with nematodes were maintained on pasture in the summer and fall. During the first two months following infection, the rotated animals gained better than the non-rotated animals. This advantage was not maintained by the calves for the remaining two months of the experiments. In general, the worm burden of the rotated animals was as great as that of the non-rotated animals. The age of the animals had a greater effect on worm burden than rotation.

Mature cattle up to the age of at least 3 to 4 years are as susceptible to initial infection with the beef measles worm as are calves. The measles persist for more than 2 years. Consequently, sanitary measures for prevention of infection with this parasite, which causes losses by condemnation and special processing of carcasses, should be employed not only during calthood, but also until the cattle go to market. (ADP bl-28)

#### H. Winter Coccidiosis (Bloody Scours) of Cattle.

Studies on this project were continued at the ADP Regional Animal Disease Laboratory at Logan, Utah. Washed sporulated oocysts of Eimeria bovis were injected either intraperitoneally, intravenously or subcutaneously into young calves in an attempt to establish an immune response. No reactions occurred in any of the calves as a result of the injections. No intestinal infections with coccidia occurred. Six weeks after these injections, oral inoculations with E. bovis oocysts were given to these calves plus a group of previously uninjected control calves. Coccidial infections developed in all four groups of calves indicating an absence of immunity in those previously injected with oocysts. Upon recovery from infection, the calves were divided into 3 groups. One group received an intramuscular injection of hormone (ACTH), and another group received an intramuscular injection of cortisone acetate. Later the 3 groups were given oral inoculations of E. bovis oocysts to challenge their immunity. There was no significant reinfection. Immunity apparently develops only as a result of intestinal infection. No changes in the blood serum potassium and sodium levels were detectable until shortly before death of the animals.

In studies to determine the number of oocysts required to produce an active immunity, 3 calves were given daily oral inoculations of 1,000, 5,000, and 15,000 Eimeria bovis sporulated oocysts, respectively, for 47 days. No significant difference was determined in the degree of immunity. Cross inoculation tests showed that severe infection with E. bovis did not protect against infection with E. zurnii.

One of two calves inoculated with sporulated oocysts of Eimeria zurnii, refrigerated at 5°C for more than 2 years, died with severe symptoms. The other calf developed less severe symptoms and survived. Cortisone acetate injected subcutaneously in young calves was used in an attempt to develop a stress method for establishing consistent infections with E. zurnii. Oral inoculations of injected and uninjected control calves resulted in inconsistent infection. (Logan, Utah)

Research workers at the Montana Veterinary Research Laboratory, Agricultural Experiment Station, Bozeman, under a cooperative agreement with the USDA, determined from observations on 8 disease outbreaks in cattle tentatively identified as "winter" coccidiosis, indicated that Eimeria zurnii was the predominant organism occurring in 4 cases, E. bovis in 3, and E. canadensis E. brasiliensis each in 1 case. Final diagnosis of clinical coccidiosis was made in only 3 instances in which E. zurnii occurred alone. A severe



case of bloody diarrhea was observed in a mature cow in which the ciliate protozoan Buxtonella sulcata apparently was the causative agent. (Montana) (ADP bl-29)

# I. Anaplasmosis of Cattle.

At the Beltsville Parasitological Laboratory, research workers reported the following findings: Ten trials to determine if females of the tick, Dermacentor andersoni, can transmit Anaplasma marginale "hereditarily," or not, were conducted using splenectomized calves. All of these trials proved negative for this type of transmission of the anaplasma from adult to larval ticks.

Agar-gel double diffusion studies revealed that precipitating antibodies do occur naturally in the serums of cattle affected with anaplasmosis. Complement-fixing antigen served as the precipitating antigen in these studies.

Electron microscopy and immunofluorescent studies have indicated that all but one isolate of A. marginale, thus far studied, have appendages which cannot be seen with conventional microscopes. This appendage assists in the identification of the parasite in preparations made from infected ticks.

A commercial, chemically purified isomer of fluorescein isothiocyanate was found to yield optimum brilliance when conjugated to immune bovine serum, which was then used to stain blood films containing A. marginale.

The resistance of carriers of A. marginale to re-exposure with the same agent was studied. It was found that some carriers withstood re-exposure, while others developed acute signs of infection.

Epizootiological studies over a 6-year period in a dairy herd in southern Louisiana have shown that acute anaplasmosis caused a 26 percent loss in milk production in lactating cows. There was no appreciable decrease in milk production of infected cows during the carrier stage.

A beef herd at the Kerrville Station, Kerrville, Texas, which in 1958 was heavily infected with anaplasmosis, has now become free of the infection. Clean heifers were used to replace their infected dams. During this year, the few animals remaining in the "reactor" herd were removed. Complement-fixation tests made at weaning time last August showed 7 reactors and 11 negative in 18 calves from the reactor cows. The 8 calves from the cows in the negative component were negative. During the February 19, 1963, complement-fixation testing, 60 days after vector peaking, one 2-year-old heifer was found to have a positive (1:10) titer. She was removed from the herd, kept in isolation and periodically retested. At the end of fiscal year 1963, she continues to give a "doubtful" reaction. At this time the

herd consists of 1 bull, 37 cows and heifers 2 to 4 years old, 9 yearling and 20 calves. A total of 383 blood samples were obtained and processed for complement-fixation testing. (Kerrville, Texas) (ADP bl-30)

Under a PL 480 grant to the School of Veterinary, Montevideo, Uruguay, research was conducted on anaplasmosis, piroplasmosis, and Babesiosis of cattle. The report of the work shows considerable progress has been made.

Bovine erythrocytes, some containing Anaplasma, and some containing Babesia, were washed and inoculated into cultures of both swine kidney cells and Hela cells. Growth of the parasites was not observed. Cellular deterioration occurred within 5 days. Hela cells were affected to a lesser degree than swine kidney cells. Cultures of splenic tissue of rats did not deteriorate following addition of erythrocytes from bovines in the clinical phase of anaplasmosis.

Electrophoretic studies were made of the serums of many experimentally anaplasmosis-infected bovines. Before anaplasms were detected in the peripheral blood, there was a decrease in the concentration of total serum proteins (TSP), of albumin, and in the albumin/globulin (AG) ratio, and a slight decrease in gamma globulin. The time when anaplasms were most numerous corresponded to the lowest level of TSP, albumin, gamma globulin, and of the albumin/globulin ratio.

Alpha and beta globulins increased during the entire course of the clinical disease, reaching their highest levels at end, 43 days, and then diminishing to pre-infection levels. The concentration of gamma globulin increased as the level of the alpha and beta globulins returned to normal. The concentration of gamma globulin was highest at the end of the study, 65 days after infection. The concentration of antibodies appears to be greater in the alpha and beta globulins than in the gamma fraction. Changes were not observed in control animals.

Anaplasmosis-experimentally-infected bovines were inoculated, intravenously, with 2 doses of Spirotripan, 20 cc per dose. Some animals were inoculated during the time the anaplasms in the blood were on the increase; others were treated during the height of the infection. These treatments were ineffectual in altering materially the natural course of the infection or of the disease.

The "mechanism of anemia" in anaplasmosis was studied in splenectomized rats, experimentally infected with Anaplasma ratti. A transient anemia, of a few days duration, was observed. Intracellular bodies in erythrocytes of 2 animals were observed. The bodies bore some resemblance to anaplasms, but could not be positively identified.

Tissue culture techniques were used to produce ovarian, muscular, and "glandular" cells of the tick Boophilus microplus for use as a media in the study of developmental stages of Babesia and Anaplasma. Only "a survival



phenomenon" occurred and, in some cultures of ovarian tissues, limited development of the ovocytes. (Uruguay) (S9-ADP-1)

J. Parasites of Cattle - Stephanofilarial Species.

This is a preliminary report on a new project initiated at the ADP Regional Animal Disease Laboratory, University Park, New Mexico. It concerns studies of worm parasites of cattle on irrigated pastures and on high-rainfall areas of the Southwest, with special emphasis on the Stephanofilarial species. Stephanofilaria stilesi has been found in 26 of 28 (93%) beef cattle examined. The lesions caused by this nematode were usually restricted to the region of the brisket in young animals and were from 1 to 2 inches in diameter, but in older cattle the lesions often extended from the brisket to the udder or scrotum, involving as much as 2 square feet of skin. Ten of 25 (40%) dairy animals examined also had lesions typical of the disease. As in beef cattle, the udder was often involved. A study of the mode of transmission of the parasite is in progress. (New Mexico) (ADP bl-33)

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DAIRY CATTLE INSECTS  
Entomology Research Division, ARS

Problem. Flies, mosquitoes, grubs, lice, and ticks are common pests of dairy cattle that cause important losses in all parts of the United States. The screw-worm, a serious pest of dairy cattle, is prevalent in Texas, New Mexico, and several western States. Heavy attacks by biting flies lower milk production by 5 to 20%. Total losses to dairy cattle attributable to insects and ticks are estimated to exceed \$200 million annually. Certain insect pests are also involved in the transmission of diseases of dairy cattle. Methods of control for dairy insects have received setbacks during recent years because the best available insecticides and most promising new materials produce residues in milk. In addition, house flies around dairy establishments have developed resistance to DDT and other insecticides. There is, therefore, great need to find safe, effective, non-residue insecticides and repellents to control these insects and ticks. Effective systemic insecticides and ways of administration which would avoid residues are needed to combat grubs in dairy cattle and to prevent the face fly and horn fly from breeding in the manure. New approaches to control, including radiation and chemosterilants, need to be explored to determine their feasibility for the control of several dairy-cattle pests. Efforts also should be made to find and evaluate insect pathogens, parasites, and predators for controlling certain dairy-cattle pests. Expanded basic studies on the biology and physiology of these pests are needed to find weak links in their life cycle to serve as a basis for the development of more effective and safer methods of control. Research is also urgently needed on the role of insects in the spread of diseases of dairy cattle.

USDA PROGRAM

The Department has a continuing program involving basic and applied research on insects and ticks which affect the health and productivity of dairy cattle. Studies are conducted on the biology, physiology, genetics and nutrition of the screw-worm fly, stable fly, horn fly, horse and deer flies, the face fly, mosquitoes, and other pests; on the nature of insect resistance to insecticides; on the mode of action of insecticides and on their absorption, metabolism and excretion by insects; the effects of irradiation and chemosterilants on insects; insect attractants and repellents; and other new approaches to control. Research is directed towards the development of more effective conventional and systemic insecticides and protective treatments for the control of dairy-cattle pests. Studies are conducted to determine the occurrence of insecticide residues in the tissues and the milk of treated animals. Minor attention is given to the development of sanitation and management procedures and to biological control, especially parasites and predators, for controlling the face fly, stable fly, horse fly, and several other pests. Studies are conducted in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions to develop physical and mechanical methods of control, to evaluate traps and devices for estimating and



controlling natural insect populations and improved or special equipment for the application of insecticides to dairy cattle. Limited research is conducted on the role of insects and ticks as vectors of animal diseases, with special emphasis on bovine anaplasmosis. The research is conducted in major laboratories at Kerrville, Tex., Corvallis, Oreg., and Gainesville, Fla., (in June, 1963, investigations were transferred from Orlando, Fla., where the research herein reported was carried out) and at satellite stations at Beltsville, Md., Stoneville, Miss., Lincoln, Nebr., and Fresno, Calif.

The Federal scientific effort devoted to research in this area totals 15.8 professional man-years. Of this number 5.6 is devoted to basic biology, physiology and nutrition; 3.7 to insecticidal and sanitation control; 2.5 to insecticide residue determinations; 0.3 to biological control; 1.9 to insect sterility, attractants and other new approaches to control; 0.3 to evaluation of equipment for insect detection and control; 0.7 to insect vectors of diseases; and 0.8 to program leadership.

## REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

### A. Basic Biology, Physiology and Nutrition

1. Mosquitoes. At Corvallis, Oreg., and Fresno, Calif., studies were continued on the biology and ecology of important mosquito species. In laboratory tests the majority of Culex tarsalis females preferred to feed at the higher of two feeding locations and preferred mice rather than chickens for their source of blood.

A study was conducted on the daily activity of several species of mosquitoes -- Anopheles freeborni, Culex peus, Culiseta inornata, Culex incidens, and Culex tarsalis. All species behaved similarly. Both males and females began to move out of daytime resting shelters a few minutes before or after sunset. Only a few males and females (0.3 to 2.8%) remained in the shelters during the night. Adults did not begin to return to the shelters until sunrise or a few minutes later, and all did not return until sometime after 8:00 a.m. Exodus from and return to shelters appeared to be regulated by light intensity. Other factors such as preconditioning to light cycles, temperature, and humidity, may have had some effect since artificial lights did not prevent mosquitoes from leaving shelters eventually, but did change their time and rate of leaving. Use of special crosses of resistant and susceptible strains of Culex tarsalis showed that some of the females mate more than one time and utilize sperm from more than one mating.

Taxonomic studies defined suitable characters for separating dark-winged forms of Aedes dorsalis from A. melanimon and determined that these two forms coexist in only one area (Solano County, Calif.). Surveys at Borrego Springs (San Diego County, Calif.) were made to find isolated populations of mosquitoes for future studies on control through sterilization.



Surveys were continued on biting arthropods of the Humboldt River Basin in Nevada. During April, May, and June, water was plentiful and high populations of Aedes melanimon, A. dorsalis, A. vexans, Culex tarsalis, and Culiseta inornata were found. Mosquito larvae were found infected with a microsporidian, Thelohania sp. and a bacterium, both of which are under study as biological control agents. In late July, no floodwater Aedes breeding was observed, but moderate breeding of Culex tarsalis and Culiseta inornata was observed. Studies of mosquito larval populations in typical breeding areas in dairy drains and culverts in California during April indicated the presence of Culiseta inornata, Culiseta incidens, Culex tarsalis, Culex peus, Culex apicalis, and Anopheles franciscanus. No breeding of Culex quinquefasciatus was found.

In Oregon, using C-14 labeled TDE, slight but consistent differences in the rate of degradation of TDE have been noted in susceptible and resistant strains of Culex tarsalis, indicating that the main source of resistance to this chemical is increased degradation of TDE to FW-152 and water soluble metabolites through an oxidative rather than a dehydrochlorination mechanism. Further tests with DDT and TDE have shown that analogs of DDT, which block metabolism via the oxidative pathway, overcome resistance to DDT and tend to confirm the hypothesis that resistance to DDT and TDE in tarsalis is due to a more rapid oxidative metabolism of the insecticide.

Early in July 1962, reports were received of a serious mosquito outbreak in the cattle-growing areas along the Gulf Coast of Louisiana and Texas. Surveys in these areas revealed a heavy mosquito population resulting from successive broods of mosquitoes over a period of 6-8 weeks. At the time of the survey, the spring calf crop had been decimated and hundreds of mature cattle had perished from the attacks of mosquitoes. Growers had sold or moved thousands of animals to avoid further losses. The loss incurred by cattlemen was estimated at \$250,000. Because of the large area involved, control of the mosquitoes was not feasible.

2. Stable Fly. In Texas stable flies began to feed when they were only 4 hours old. The adults did not feed well or survive long on deer blood or several synthetic diets. They thrived on beef blood and beef serum but not on blood cells alone. At 80°F. and 50-60% relative humidity first matings occurred when the flies were 3 days old and 84% mated within 5 days. At 80°F. and 10-30% relative humidity mating did not begin until flies were 4 days old and only 12% mated in 5 days. No mating whatever occurred at 68°F. and uncontrolled humidity.

Stable fly larvae were not adversely affected by 17 hours of submergence in water, but 100% mortality resulted from 24 hours submergence. Exposure of pupae in water for 17 hours resulted in retarded development and greatly reduced adult emergence.



In Oregon field studies determined the dispersal pattern of marked adult stable flies. Flies were recovered over 14 miles from the point of release in 24-48 hours.

3. Face Fly. In Nebraska, face flies were first observed on cattle in the field on April 25 and by mid May they were present in annoying numbers. By early June heavy populations of 20 or more per head were widespread.

Studies on the behavior of the face fly were conducted in a large outdoor screened cage in which a calf was restrained. Several hundred laboratory-reared flies were released in the cage before each observation period. Less than 4% of the flies were present on the calf at any one time. The remainder rested in the grass or on the sides of the cage. Only an occasional male was observed on the calf. Nearly half of the flies congregated around the eyes and about 25% on the muzzle.

4. Horn Fly. In Texas, after considerable trial and error, a technique was developed for colonizing the horn fly in the laboratory without access to a bovine host. The diet consisted of 1 part bovine blood and 1 part beef juice plus 1 mg. of streptomycin, 1000 units of penicillin and 250 units of mycostatin per ml. of diet. Flies were fed by placing a cotton pad soaked with the diet on top of the holding cages. Cages consisted of 4" lengths of 6" plastic tubing with ends of plastic screen. Eggs were deposited on the plastic screen. Optimum conditions for adult reproduction were 90°F. temperature and 60-80% relative humidity. The colonization procedure is easy and inexpensive and greatly facilitates laboratory testing of insecticides, chemosterilants and repellents at all seasons of the year. Adult flies survived for 96 hours in a room kept at 75-80°F. and 62-67% relative humidity. Only a few survived in a room kept at 85-92°F. and 34-40% relative humidity.

In Oregon field studies determined the pattern of dispersal of marked adult horn flies. Flies were recovered over 7 miles from the point of release in 24-48 hours.

5. Screw-worm. Research on the screw-worm was discontinued at Kerrville, Tex., in September 1962 and moved to Mission, Tex., headquarters of the Southwest eradication campaign. This included research on the biology and ecology of the screw-worm under field conditions, vigor and longevity of sterilized flies, effects of radiation, the development of genetically marked strains, cytological studies with irradiated flies, nutrition, and special tests related to problems in the sterile male release program.

A new technique to determine the mating aggressiveness of sterilized or mutant screw-worm flies was developed. It was observed that in laboratory cages males harassed females sufficiently to cause mortality greater than that occurring when females were caged without males--in general, higher ratios of males to females caused greater mortality than lower ratios. Tests with various strains of screw-worms of known sexual competitiveness



in comparison with other mating aggressiveness tests confirmed the validity of the method. Since the criterion for mating aggressiveness is the mortality of females over time rather than egg production and viability, the time, space, and labor required to determine sexual aggressiveness were greatly reduced. Studies confirmed optimum numbers of screw-worm larvae per tray for rearing efficiency and optimum numbers of adults per cage for mating and longevity studies. They also confirmed diurnal periodicity in screw-worm pupae for emergence occurred from sunrise until noon. Studies indicated that the use of  $\text{CO}_2$  as anesthesia for screw-worms is safe for handling young screw-worm adults for longevity and mating tests and scanning for genetic markers.

Research was continued to find and develop genetically distinct strains of screw-worm flies and to study these mutant flies. In the adults many genetic markers were found such as yellow eyes and white auxillary region ("Whaxy"). The genetic marker "Whaxy" affects the morphology of both the adult and larvae--a factor which would be invaluable in field studies. Approximately 270,000 screw-worm larvae were examined for morphological variants and from this 221 cultures were studied as possible genetic strains. Of markers found in the immature forms, at present three morphological characters--interrupted bands of segmental spines, two spiracular openings in the anal plates of third-instar larvae, and additional spines on the eleventh anterior segmental band--were demonstrated to be genetic in nature. None of these were established as a pure strain. Research with these and other mutant strains is being pursued to determine the genetics of the strains and if these strains are as vigorous and aggressive as the normal strain, factors which are essential if the strains are to be used in the release program. For example, preliminary genetic studies indicate that the "Whaxy" mutant is lethal in the homozygous state with only a few individuals escaping. Other strains have slight behavioral differences from the normal strain, i. e., they respond differently to attractants and survey traps.

When pupae of the screw-worm were irradiated in an atmosphere of  $\text{CO}_2$  and air (50-50 mixture), damage to the reproductive system of the adult female was greater than that induced by a similar radiation treatment in air. When the pupae were pretreated in the gas mixture for approximately 45 minutes, complete sterility was induced by a treatment of 4,500 r delivered in  $\text{CO}_2$  and air, whereas irradiation in air alone required about 5,500 to 6,200 r. Techniques were developed to study the cytological effects of radiation and chemosterilants on screw-worms, particularly on spermatogenesis and oogenesis. Preliminary studies revealed chromosomal aberrations severe enough to cause dominant lethality. Considerable data on oogenesis and spermatogenesis in normal screw-worm flies were accumulated as necessary background data for evaluating the effects of radiation and chemosterilants on reproduction.

For rearing larvae of the screw-worm, a synthetic diet was developed that contained casein, yeast extract, cholesterol, inorganic salts, water, and



agar. This medium was further defined by replacement of casein with a mixture of L-isomer amino acids, and of yeast extract with a mixture of B-vitamin and RNA. Larval growth and development on the defined medium were nearly equal to that on media containing casein and yeast. Larvae absolutely required thiamine, riboflavin, panthothenate, niacin, and choline for growth. Biotin and folic acid stimulated growth and were necessary for maturation to the adult stage. Pyridoxine and its analogs, pyridoxal and pyridoxamine, inositol, B<sub>12</sub>, and carnitine had no effect on growth. Niacinamide spared niacin, but p-aminobenzoic acid had no effect on the folic acid requirement.

Studies on the effect of dessication, lack of adult food, reduced temperature, age of flies at time of release, and the effect of all of these factors on different strains of the screw-worm were undertaken to improve the survival of screw-worm flies released into the hot and dry climate of Texas. Provision of food in release cartons or the judicious use of reduced holding temperatures curtailed mortality prior to or shortly after release. Techniques need to be developed which would allow greater uniformity in age of flies at the time of release. Newly emerged flies carry a food and water reserve that will sustain them up to 24 hours (90°F. and 30% R.H.) compared to only 3 to 4 hours for 2-day-old flies. Selection of flies for individuals more capable of survival under unfavorable conditions has shown promising results.

Extensive studies were made of the ecology of screw-worm flies under field conditions by releasing tagged flies. It was determined that flies tend to congregate and disperse along water courses or streams and are capable of traveling long distances. This research resulted in the addition of strategic releases of screw-worms along water courses and an increase in the effectiveness of the sterile-male release program.

6. House flies. At Orlando, Fla., research on control or eradication through the use of sterilization by radiation or chemicals has shown that considerable gaps exist in knowledge of the biology and mating behavior of house flies. Mating tests have shown that both males and females must undergo a sexual maturation time of at least 16 hours with males and a minimum of 24 hours with females. Once the mating drive has started, males will attempt to "strike" or mate with both males and females and certain inanimate objects, although they "strike" more readily and frequently with females. This fact along with experiments in a large cage-type chemotactometer suggested the presence of some type of a female sex attractant of a low order. Imperfect sex recognition in the male combined with a low order sex attractant in the female would account for the fact that males attempt to mate with either sex and result in the higher ratio of male-to-female "strikes." Quantitative data on the male and female mating behavior and a detailed description of the mating "strike" of the male and mating behavior of the male and female have been developed. Males or females which had their wings removed were able to mate with individuals of the opposite sex



with wings. Amputation of more than one pair of legs from the male prevented mating, while amputation of only one pair of legs impeded but did not prevent mating.

Tests were run to determine the actual time house flies remain in copula after initial seizure. Of 61 mating pairs that were trapped and observed, the shortest mating period was 44 minutes and longest, 96 minutes. The average was 60 minutes. Females examined after only 1 and 2 minutes of mating contained no sperm. Those examined after 3 to 5 minutes of mating had spermathecae which contained a few sperm and some which were completely filled. After mating for periods of 10 to 76 minutes, the spermathecae were filled to capacity with sperm, with only one exception.

Eighty-six female house flies reared in the laboratory laid an average of 94.4 eggs per female of which 89% hatched. The number of eggs laid varied from 12 to 186 per female. Under laboratory conditions some flies fed within an hour after emergence; others did not feed until later and some did not feed until they were 16 to 18 hours old.

At Corvallis, Oreg., studies were continued on the physiology of resistant and susceptible house flies. Isolan-resistant house flies have decreased ali-esterase activity and increased Isolan-detoxifying enzyme. Techniques using high-speed centrifugation have been developed which concentrate enzymes important in metabolizing or conferring resistance to organo-phosphorus compounds. In the case of malathion-resistant house flies, the nature of the alkyl groups attached directly to the phosphorus atom is the major factor in resistance. Thus, the primary cause of resistance is probably associated with the rate of recovery of the phosphorylated detoxifying enzyme after poisoning, rather than with an increased ability to cleave the toxic molecule per se.

In Oregon extensive studies were conducted on the biology, nutrition, and colonization of the little house fly (*Fannia canicularis*). After much trial and error a satisfactory rearing medium was developed which consisted of alfalfa meal, yeast extract solution and wood shavings. Adult flies were held in standard cages and provided with wrinkled balls of black muslin impregnated with alfalfa and yeast extracts for oviposition sites. Under such conditions 48% of the females and 25% of the males survived for 22 days in cages kept at 70-80°F. At 90°F. no males and only 8% of the females survived for 22 days. Egg deposition was about 5 times as great at 80°F. as at 70° or 90°F. Females began ovipositing in 5-6 days at 90°F., 8-9 days at 80°F., and 10-11 days at 70°F. The eggs dessicate rapidly and must be kept constantly on a moist surface to insure a high percentage of hatch.

7. Cattle Grubs. Research was continued in Texas and Oregon to determine the nutrition requirements of cattle grub larvae and to develop an artificial medium for rearing larvae under laboratory conditions. In Texas



various methods and media were tried in an effort to rear grub larvae. First stage larvae survived as long as 13-15 days in several tests but no second and third instars survived more than a week.

In Oregon, studies were conducted to determine the optimum conditions for larval pupation and development. Pupae held in moist sand at 40°, 44° and 50°F. produced no adults. Some emergence occurred at constant temperatures of 60° and 68°F. and at fluctuating temperatures ranging from 63°-75°F. Emergence was greater under fluctuating temperatures than at constant temperatures. Examination of pupae indicated that failure of adults to emerge was due to inability to eclose.

8. Horse Flies and Deer Flies. Studies were continued in Mississippi on the biology of tabanids. First adults of Hybomitra lasiophthalmus were noted on March 28. By mid-April this species was abundant and small numbers of T. vittiger schwardti and T. fuscicostatus were present. The usual succession of species appeared during the summer and early fall months but none reached their usual abundance. Population declined drastically during the latter part of July as a result of prolonged drouth. Populations rose only slightly following rains in late August.

9. Lice. Studies in Oregon showed that low louse populations on cattle during the summer months are largely due to destruction from licking. Short summer hair coats do not protect the lice, whereas long winter coats do. When short coated animals were restrained from licking themselves, heavy louse populations developed in 3-4 weeks.

10. Ticks. Observations in Texas showed that the lone star tick became active in January 1962, and population on cattle increased gradually during February, March and April, reaching a peak in May and June. Tick populations began to decline in July and had virtually disappeared by September.

The winter tick first appeared on cattle in October 1962, and populations gradually increased to a peak in December 1962, and January 1963. Tick populations declined rapidly in February and had virtually disappeared by late March.

## B. Insecticidal and Sanitation Control

1. Mosquitoes. Studies were continued at Orlando, Fla., to find new and effective insecticides for the control of mosquitoes. In screening tests with Anopheles quadrimaculatus larvae, 28 of 81 compounds tested were rated Class IV in effectiveness. Four of these compounds--American Cyanamid CL-18133, Stauffer B-10046, Stauffer 8-10094, and Bayer 47940--were highly effective, killing 100% of the larvae at concentrations of 0.05 to 0.01 p.p.m. One hundred and nine plant extracts were also screened for toxicity, but none of these were toxic at low concentrations. Of 83 compounds tested against female Aedes taeniorhynchus in wind tunnel tests, 7 were as effective as the standard, malathion. Dimethrin in granular formulations was effective



in laboratory and field tests as a larvicide against Anopheles quadrimaculatus, Aedes aegypti, Aedes taeniorhynchus, and Culex quinquefasciatus mosquitoes. A mixture of DDT and an anti-resistant compound was no more effective than DDT alone against C. quinquefasciatus larvae.

In Oregon, studies were continued to find repellents for protecting animals from attacks by mosquitoes. Only one (ENT-26455) of 91 compounds tested was a highly effective repellent. This material showed 100% repellency for 24 hours and from 50 to 90% repellency after 48 hours. Several materials were highly effective toxicants but none was superior to the standard, Bayer 29493.

In Oregon, 46 compounds were tested for systemic action by giving them orally to mice and allowing mosquitoes to feed on the mice. At a dosage of 100 mg./kg., two compounds--Bayer 29493 and Shell SD 8436--killed all mosquitoes for 6 hours after the mice had been treated.

2. Stable Fly. In Texas, 240 compounds were screened in spot tests on cattle for repellency and toxicity against the stable fly. Of these materials, 6 were Class IV repellents at 5% and 21 were Class IV toxicants at 0.5%. A number of other materials were Class IV at higher concentrations. The outstanding repellents were ENT-20274, ENT-25927, ENT-26864, ENT-32965, ENT-25946, and ENT-27031.

Tests were conducted with the WHO test kit to determine the susceptibility of non-resistant stable flies to DDT and dieldrin. At an exposure of 30 minutes the LD-50s and LD-90s for DDT were 1.7 and 2.6% and for dieldrin 0.36 and 0.91%, respectively.

In field tests in Nebraska, applications of 1 pint of 0.5% Ciodrin to cattle gave good control of stable flies for 3-4 days. Daily applications with an automatic sprayer of 100 cc of 0.8% Ciodrin maintained very good control of stable flies. In field tests in Texas, sprays of 0.25% DOWCO 175 (ENT-25964) applied at the rate of 2 quarts per animal gave excellent control of stable flies for 3 days.

3. Face Fly. In Nebraska, tests were conducted in which materials were administered to cattle in feed in order to determine their effectiveness in preventing fly breeding in feces. Concentrations of 0.1% and higher of Bacillus thuringensis, an insect pathogen, prevented fly larval breeding in droppings. At concentrations of less than 0.1% larval survival increased in proportion to the decrease in concentration. Phenothiazine (2.5-3 microns) administered at the rate of 2 grams daily for 12 days gave erratic results but in no instance provided effective control of larval breeding. A concentration of 20 p.p.m. of Co-ral in feed completely inhibited larval breeding and DDVP at 0.5 mg./kg. was almost completely effective. Free choice consumption of salt containing 0.5 or 1.0% Co-ral gave erratic results, larval



control ranging from 40 to 85%. The erratic results were attributed to differences in consumption (0.06 to 0.35 mg./kg. daily) by individual animals.

In Nebraska, tests were conducted in which 4% malathion and 0.8% pyrethrins plus 1.1% piperonyl butoxide were installed around salt boxes. Daily contact with the dust bags caused reduction in face fly populations on cattle.

4. Horn Fly. Extensive field tests were conducted in Texas, Mississippi, Nebraska, and Oregon, to compare several old and promising new insecticides for the control of horn flies on cattle. In Texas sprays of 0.25 and 0.5% Hooker 1422 (ENT-25780) applied at the rate of 2 quarts per animal provided effective control of horn flies for 7-10 days. Applications of 2 quarts of 0.5% Ciodrin and 1 pint of 2% Ciodrin provided 14-95 days control in dry central Texas and 11-12 days control in humid east Texas. Tests were conducted with several materials applied in minimal amounts by an automatic mist sprayer. Daily applications of 0.2% or 0.5% of DDVP provided excellent immediate control and kept animals entirely free of horn flies. Spraying on alternate days kept populations at a sub-annoying level. Similar results were obtained by spraying daily with 0.125-0.25% Ciodrin. Single applications of 0.5, 0.75, and 1.0% Ciodrin provided effective control for 10, 14, and 14 days, respectively.

In Mississippi, sprays of 0.5 or 1.0% malathion applied at the rate of one-half pint per head provided excellent control for 4 days and satisfactory control for about a week. Similar applications of 0.5% Cygon, methoxychlor, toxaphene and Famophos were effective for 3, 4-5, 4-5, and 5-6 days, respectively. Applications of 2 quarts per animal of 0.5% toxaphene or Co-ral provided effective control for 3-5 and 11 days, respectively. In tests with automatic sprayers, applications of 6 ounces (3 round trips per animal) in one day of 0.1% Baytex, 0.15% Delnav, 0.5% malathion, 0.5% toxaphene, 0.5% Bayer 22408, 1.0% Ciodrin, and 1.0% Ciodrin plus 0.25% DDVP provided effective control for 5-6 days. Ronnel and Baytex at 0.5% were effective 3-4 days. Similar applications of 0.1% Baytex, 0.5% Cygon and 0.5% Bayer 22408 were effective 1-2 days.

In Nebraska, 2 quart applications of 0.1 and 0.25% Stauffer R-1504 (ENT-25705) gave good control of horn flies for 11 days.

Tests were conducted to determine the effectiveness of low-level feeding of a number of materials in preventing horn fly breeding in cattle droppings. In Texas, daily feeding of Bayer 29493 at 1 mg./kg., Bayer 37341 at 2.5 mg./kg. and Bayer 37342 at 5 mg./kg. gave 100% control of larval breeding. Three commercial brands of Bacillus thuringensis administered at concentrations of 0.05-0.1% in feed also prevented fly breeding in droppings. These materials gave similar results in free choice feeding tests but the effective concentration of two of the materials was about 4 times that of the third one.



In field tests in Oregon, one-quart applications of 1% Shell SD-8436 (ENT-25840) gave highly effective control for over a week and 0.25% was moderately effective for this period. Similar applications of 0.25% Ciodrin, Bayer 29493, and Famophos, and 0.5% malathion, were effective for 4 days. Five insecticides were evaluated at high concentrations applied at low volumes (12 ml) to the backs of cattle with a pump oil can. In these tests 4% DDT was effective more than 21 days, 4% methoxychlor for 11-15 days, 4% malathion for 7-9 days, 4% Barthrin for 7 days and 5% Barthrin for 14 days. In feeding tests, single oral administrations of 50 mg./kg. of ENT-25842 inhibited fly breeding in feces for 6-9 days. A similar dose of ENT-25840 was effective 4-5 days. A dose of only 1 mg./kg. of either compound was effective for 1 day. In tests involving feeding over a period of 5 days Zytron at 5 mg./kg. or carbophenothion at 10 mg./kg., prevented breeding in feces the first day. Lower doses of these materials and Bayer 29493 inhibited breeding after 3 days. Famophos 20 mg./kg. and Stauffer R1504 at 10 mg./kg. were not completely effective.

6. House Flies. Research was continued at Orlando, Fla., to develop more effective insecticides and other methods and materials for the control of house flies. Twenty-three new compounds were tested as space sprays in a wind tunnel against the regular susceptible colony and the Cradson (multiresistant) colony. Fourteen of the compounds were more effective against both the susceptible and resistant colonies than the standard, malathion. New insecticides were also evaluated as residual treatments against female house flies from the regular or Cradson colonies. The criterion of effectiveness was the number of weeks of aging during which the residues remained effective in killing house fly females. Against susceptible house flies Hercules AC-5727 alone and Bayer 39007 alone gave kills of 90% for one week, but in combination with Monsanto CP-16226, their effective periods were extended to 8 weeks and 12 weeks, respectively. The effectiveness of Hercules 7522H was also extended from 1 to 12 weeks with the addition of Monsanto CP-16226. Against flies of the Cradson colony all of these formulations failed before the fourth week. Against the regular colony Bayer 29952 and Bayer 30237 were effective for 64 and 56 weeks. Stauffer N-2230 and Stauffer N-2404 were 100% effective throughout 48 weeks of aging against the susceptible colony, but they were ineffective against the Cradson colony. General Chemical GC-3583 was still 100% effective after 96 weeks against the regular colony and Monsanto CP-40294 was effective for 24 weeks against the Cradson colony. Against house flies from the susceptible colony, General Chemical GC-4072 was 100% effective for 96 weeks as an acetone solution and Stauffer N-2310, Bayer 39197 and Monsanto CP-40273 for 48+ weeks. As wettable powder DDT was more than 90% effective for 48 weeks, Bayer 25141 for 48 weeks, Bayer 34098 for 40 weeks, Hooker HRS-1422 for 32 weeks, and Bayer 32651 for 28 weeks.

Residual tests (deposits of 100 mg./sq. ft.) were conducted with emulsions of diazinon, Baytex, and dimethoate against house flies in Florida dairy barns. The diazinon treatment failed to give satisfactory control as early



as the first day after treatment. Baytex gave 97% control of 5 days and from 79 to 88% control through 14 days. Dimethoate gave controls ranging from 80 to 96% for 6 weeks, when the test was discontinued.

At Corvallis, Oreg., extensive studies were continued on the development of synergists that have been shown to overcome resistance to organophosphorus insecticides in both house flies and mosquitoes. Of some new types of compounds screened, results indicate that diisopropyl or dibutyl substitutes would be most satisfactory. Selection of house flies with combinations of malathion and synergists are being carried out to determine if resistance to the combinations can be developed.

7. Cattle Grubs and Other Bots. Research was continued in Texas and Oregon to develop more effective insecticides for controlling cattle grubs and other bots affecting livestock. In Texas 79 new compounds were screened for systemic action by giving them orally (O), subcutaneously (SC) or intramuscularly (IM) at several dosages to guinea pigs infested with larvae of the screw-worm, Cochliomyia macellaria or Phormia regina. Nine of the materials showed systemic action in one or more types of administration. The active compounds and minimum effective dosages in mg./kg. were as follows: Stauffer R10094, 15 mg. O and 25 mg. SC; Shell SD-8448, 50 mg. SC; Shell SD 8530, 100 mg. O and 50 mg. SC; Chemagro S-8550, 100 mg. O; Hercules 7845, 10 mg. O and 25 mg. SC; Stauffer R-6032, 25 mg. O; Monsanto CP 19203, 10 mg. O and SC; Shell SD 8280, 10 mg. SC; and Pyramat, 100 mg. SC.

In Texas tests were conducted on small numbers of animals (2-4) with a number of compounds that had shown promise in screening tests or on individual cattle in 1961-1962 and with older effective materials administered in different ways. The effective materials, dosage, and route of administration were as follows: Co-ral, 2 and 8% pour-on; Bayer 37341, 2% pour-on; Bayer 37342, 2% pour-on, and 15 mg./kg. IM; Famophos, 20 mg./kg. IM; Stauffer R1504, 2% pour-on and 2.5 mg./kg. in feed for 10 days; Rhodia, 15 mg./kg. in feed for 10 days; Dipterex and Butonate, 5 mg./kg. in feed for 10 days; and Baytex, 2.5 mg./kg. in feed for 10 days. All of these treatments gave 97-100% control of the two species of grubs which infested the Wyoming cattle used in these tests.

Extensive field tests with Government and cooperator herds of cattle were conducted in Texas and Oregon to evaluate the effectiveness of promising new and several older systemics at different rates and various methods of administration. In Texas, a total of 30 materials were tested but only 7 produced 89-100% control. The effective materials, dosage and methods of application are as follows: Baytex, 1 mg./kg. in feed for 10 days; Dipterex, Butonate, and Bayer 37341, 2.5 mg. in feed for 10 days; Bayer 37342, 5 mg. in feed for 10 days; Famophos, 12.5% pour-on (65-125 mg. per animal); Imidan, 2% pour-on (125 mg.) and 10 mg./kg. IM.

In Oregon extensive tests were conducted with eight known systemics with the primary objective of determining the minimum effective dosage by pour-on and



spraying. In pour-on tests, applications of 82 and 120 ml. of 12.5% Famophos in oil or glycol gave 99-100% control of grubs. Lesser amounts of these concentrations were slightly less effective (91-92% control). Oil applications of Famophos were slightly more effective than emulsions. Pour-on applications of 60 and 120 ml. of 12.5% Bayer 37342 gave 95 and 96% control. With Bayer 29493 as little as 20 ml. of 10% in oil and 40 mg. of 5% in oil gave 92% control of grubs. Pour-on applications of 5 grams of Shell SD-8436 and 20 grams of Shell SD-8448 gave 100% control of grubs. Oral administrations of 50 mg./kg. of both compounds failed to give satisfactory control.

In a series of spray tests, one gallon applications of 1, 1.5 and 2% Dipterex gave 97-100% control of grubs. Lower concentrations did not give satisfactory control. One-half percent Ruelene sprays produced 93% control but at 0.25% control was only 80%. Sprays of Co-ral at 0.5% gave satisfactory control in only 1 of 3 tests. Bayer 29493 gave 100% control as a 0.25% spray but did not give satisfactory control at concentrations of 0.1 and 0.05%. A series of tests was conducted to compare the effectiveness of Famophos, Bayer 37342, Bayer 29493 and Ruelene at different dosages on a "per animal" basis rather than on body weight estimates. In these tests minimum dosages of Famophos at 10 grams per head, Bayer 37342 at 5 grams per head, Bayer 29439 at 2 grams per head and Ruelene at 2 grams per head provided 98-100% control of grubs in cattle of mixed sizes and ages. These results indicate that dosage applied on a per-animal basis is as satisfactory as dosages applied on body weight estimates.

8. Horse Flies and Deer Flies. In Mississippi tests were conducted to determine the effectiveness of synergized pyrethrum dusts containing 0.1 and 0.067% pyrethrins. Both formulations gave excellent protection from horse flies the day of treatment and fair protection for several days. Mist spray applications of 2 ounces of activated pyrethrum containing 0.62% pyrethrins were highly repellent to horse flies for 6 hours. Lower concentrations were proportionally less effective. Similar applications of 5% ENT-21195 provided complete protection for 6 hours and afforded 79% protection after 24 hours.

9. Lice. In field tests in Mississippi, eradication of cattle louse infestations was achieved with a single spraying of 0.5% General Chemical 4072 and 1% Sevin. Similar results against hog lice were obtained with sprays of 0.1 and 0.25% of GC 4072, 0.5 and 1.0% of Sevin, 0.25% of Ciodrin and 0.5% of Ciodrin, Dilan, and methoxychlor.

10. Ticks. Research on the control of ticks was confined to the Texas laboratory. Only 5 of 79 compounds screened for systemic effectiveness showed systemic action against lone star ticks engorging on treated guinea pigs. The effective materials, dosages (mg./kg.) and routes of administration were as follows: Monsanto CP-19203, 10 mg. O; Hercules 7845 and Stauffer R6032, 50 mg. O and SC; Shell 8280, 50 mg. SC; and Shell 8530, 100 mg. O.



In field tests, sprays of 0.5% toxaphene and 0.25% Stauffer 1504 gave excellent control of lone star ticks on cattle and were slightly better in both immediate and residual effectiveness than 0.025% diazinon, 0.05% carbophenothion, 0.1% Ciodrin and 0.25% Sevin and Dilan. In tests against the winter tick, complete control was obtained with sprays of 0.05% carbophenothion, 0.25% Bayer 37341, 0.1% Dipterex, 0.25% Dowco 175, and 0.3% Ciodrin. Diazinon at 0.05% gave excellent but incomplete control.

### C. Insecticide Residue Determinations

1. Residue Studies. Studies were conducted in Texas on the absorption, distribution, storage and metabolic fate of insecticides in animals using chemical and radiometric methods of analysis. In Texas, steers were sprayed with 0.05 and 0.1% diazinon 1, 2, 6, and 10 times at weekly intervals and residue determinations made 6 days after the last spraying. One and two sprayings of 0.05% diazinon produced residues of only 0.05-0.09 p.p.m. in the fat. Six and 10 sprayings produced residues of 0.20-0.35 and 0.17-0.23, respectively. However, one day after the 10th spraying residue levels ranged from 0.53 to 0.85 p.p.m. Animals sprayed with 0.1% diazinon showed residues ranging from 0.47 to 0.83 p.p.m. There was very little difference in the residue levels created by 1, 2, 6, and 10 sprayings.

A Hereford calf was sprayed with  $C^{14}$ -labeled Stauffer R-1504 at a rate equivalent to 2 quarts of 0.5% Stauffer R-1504. The calf was slaughtered 7 days after spraying and samples of tissues were taken for analysis. Analyses were made by a radiometric method and by the chemical sulfide method. Residues found by the sulfide method were 0.04 p.p.m. in the omental fat, 0.11 p.p.m. in the subcutaneous fat, and less than 0.02 p.p.m. (sensitivity of method =  $\pm$  0.02 p.p.m.) in other tissues. The radiometric method (sensitivity of method =  $\pm$  0.001 p.p.m.) gave values of 0.030 p.p.m. in the omental fat, 0.41 p.p.m. in the subcutaneous fat, 0.021 p.p.m. in renal fat, and 0.001 to 0.004 p.p.m. in the other tissues.

Residue determinations were made from 3 calves slaughtered 7, 16, and 28 days after spraying with 0.25% General Chemical 4072. The amounts of General Chemical 4072 found in the various tissues ranged from 0.004 to 0.0085 p.p.m. 7 days after spraying, 0.004 to 0.006 p.p.m. 16 days after spraying, and in all cases were 0.005 p.p.m. 28 days after spraying. A still unidentified material appeared in the liver of the calf slaughtered 7 days after treatment, and also in other tissues of the calves slaughtered 16 days and 28 days after treatment. It is possible that this material is a metabolite of General Chemical 4072.

2. Toxicity Studies. Work was conducted in Texas in cooperation with veterinarians of the Animal Disease and Parasite Research Division on the acute and chronic toxicity of insecticides and other materials. A summary of the results are presented. Detailed results will be given under Unit 2, Animal Diseases and Parasites (ADP a7-12, ADP a7-18, ADP a7-19, ADP a7-20, and ADP a7-23).



A colorimetric analytical method for studying the toxicology and presence in animal tissues of 2,4-D was successfully developed by using Carbon-14 labeled 2,4-D which could be determined with radiation detection instruments at each step of the developing technique. The analytical method which resulted is capable of detecting 0.05 parts per million of 2,4-D in animal tissue samples weighing 25 grams.

The ordinary feeding of Vitamin A to cattle increased their susceptibility to poisoning by Co-ral, particularly when they were also given phenothiazine drenches for internal parasite control. When impurities appeared in Co-ral, as they did during the fall of 1962, the toxicity was even greater. However, contaminated Co-ral, such as was credited with causing losses of cattle exceeding \$750,000 in value, could not be shown to produce poisoning in Kerrville cattle unless Vitamin A and phenothiazine were also used. These initial studies need to be followed with others to determine whether the effect is limited to Co-ral or also follows the use of other insecticides.

More important than the increased incidence of poisoning when the Vitamin A and phenothiazine are present is the type of poisoning produced. Whereas Co-ral can poison and kill any animal, its activity normally is against an essential enzyme, cholinesterase. Poisoned animals that do not die rarely show any appreciable tissue changes. In the poisoning observed this year, there was a marked tissue change. One of the changes noted was a necrosis (death) of muscle fibers, particularly of the thigh muscles that compose the "round" meat cuts. In the living animal the necrosis is apparent as a lameness, in the carcass as an area of "white muscle" showing clearly against the normal red.

Four important enzyme systems were affected by the Vitamin A - phenothiazine - Co-ral combination. The significance of the effect is not yet clear.

Brahman cattle, and their crosses, were more susceptible to poisoning by Ciodrin and Compound 4072 than European breeds and their inter-crosses. The blood enzyme, cholinesterase, was more readily inhibited in Brahman and their crosses than in European breeds. With Compound 4072, the only chemical given both by mouth and as a spray, the susceptibility was greater in Brahman by both routes, indicating that the peculiarities of the Brahman skin were secondary to the species difference. The increased susceptibility was most marked to the compound Ciodrin.

In studies of the detoxication mechanisms in cattle and sheep, oximes, including 2-PAM chloride, DAM, and P<sub>2</sub>S, were used to counteract poisoning by various organophosphorus compounds. The oximes are useful to cause a release of the enzyme (cholinesterase) inhibited by this group of pesticides. All three compounds were effective in mild or moderate poisoning. Their action was somewhat slow and atropine sulfate was still required in severe poisoning to gain time for the oximes to work.



Sodium selenite, sodium selenate, and d-alpha tocopheryl (Vitamin E) were effective in several instances of organophosphorus poisoning in our research. Studies are needed to explore the mechanisms by which these two substances exert their beneficial effects.

Thirty-two insecticides, most of them currently under test against livestock insects, were studied during the year. These studies furnished toxicological guidelines for decisions for further development of the materials. Co-ral and arsenic did not show potentiation in cattle and calves when used on the same day or within one or two days of one another. The study was conducted to show the safety of using both compounds on cattle imported from Mexico. Sprays of toxaphene following Co-ral sprayings seemed to reactivate the Co-ral deposits, leading to mild Co-ral poisoning in cattle and calves so treated.

Three fungicides and thirteen herbicides were studied in cattle. Generally, except for the mercurial fungicides, massive dosages repeated on several days were required to produce poisoning. Four herbicides, simazine, atrazine, bandane and promazine afflicted the nervous system. With bandane, a yearling steer died of a cerebral and medullary hemorrhage after showing various degrees of paralysis and other neurological symptomatology.

#### D. Biological Control

1. Mosquitoes. At Fresno, Calif., in cooperation with the Bureau of Vector Control, California State Board of Health, studies were initiated on the biological control of mosquitoes. Several species of microsporidia of the genus Thelohania killed mosquito larvae. Studies are under way to determine the host-parasite relationship.

2. Stable Fly and House Fly. Spalangia muscidarum, a pupal parasite that attacks stable flies, house flies and other Diptera, was reared in sufficient numbers in the laboratory at Lincoln, Nebr., to permit the start of systematic releases on May 16. An area of 16 square miles containing 20 farms was selected as a release area and 5 farms at another location were used for control observations. The release rate was varied from farm to farm according to the extent of the potential fly breeding areas. The release of this parasite, plus the natural occurrence of several other parasites, failed to produce marked reductions in stable fly or house fly populations. The percentage parasitism of pupae was erratic but at times very high. In general, planted pupae were more heavily parasitized than naturally occurring pupae. The percentage parasitism ranged from 0 to 100, without any apparent correlation with either time of year or the number of parasites released. In the course of these studies it was determined that three parasites parasitize stable fly pupae only, four parasitize both stable fly and house fly pupae, and four parasitize house fly pupae only.



3. Face Fly. In Nebraska observations were made on the effect of dung beetles on face fly larval breeding in cattle droppings in pastures. The constant movement of the beetles through fresh droppings reduced larval populations by about 80%.

#### E. Insect Sterility, Attractants and Other New Approaches to Control

1. Mosquitoes. In Oregon tepa and ENT-50450 were tested as sterilants against Culex tarsalis adults by spraying in wind tunnel tests and against larvae by exposure in water. Tapa sterilized males and females at a spray concentration of 6%; ENT-50450 sterilized only males at this concentration. Lower concentrations of either compound were not effective. Against larvae, both compounds were toxic at higher test concentrations and ineffective and partial toxicants at lower concentrations.

In Oregon studies have shown that grass infusion and log pond waters are attractive to ovipositing female Culex pipiens quinquefasciatus. Distilled water treated with methane or furfural was more attractive to ovipositing females than distilled water, but less attractive than log pond water.

2. Stable Fly. In Texas a small number of chemicals was evaluated by several methods as chemosterilants against the stable fly. Topical applications of 1.0 ug of ENT-26382 sterilized both sexes of flies. Similar applications of 4 ug of ENT-50569 sterilized males but did not completely sterilize the females. Three other materials--ENT-50042, 50396 and 50549--at 4 ug per fly, reduced oviposition and hatch but did not fully sterilize either sex.

In feeding tests, flies were completely sterilized when fed baits containing 0.2% aphoxide for 24 hours. Flies fed 0.1% aphoxide bait produced normal numbers of eggs but only 2% hatched. Several materials were tested as residual treatments but none of them produced complete sterility.

Studies were conducted to determine the absorption, metabolism and excretion of a  $P^{32}$ -labeled chemosterilant (MAPO) applied topically and in the diet of stable flies. When applied topically, the material was rapidly absorbed, with the females showing maximum absorption in 6 hours and the males in slightly longer time. The material was metabolized to the extent of about 60% in 24 hours. The main metabolic products were phosphoric acid and an unknown intermediate. Four other metabolites were isolated but not identified.

In the feeding tests MAPO was absorbed and distributed slower and in lesser amounts than in the topical tests. After 24 hours the degree of metabolism was about 50% higher than in the topical tests. In contrast to the topical tests, the main metabolite was an unknown intermediate which was about 4 times as abundant as phosphoric acid. Three of four other unknown intermediates occurred in greater amounts than phosphoric acid. Only about 8% of the applied dose of MAPO was excreted.



3. Face Fly. In Nebraska improved techniques were developed to screen materials as attractants for the face fly. Over 200 inorganic and organic materials were tested but none was as attractive as the liquid portion of fresh cattle manure.

A number of known chemosterilants was evaluated for effectiveness against the face fly. Flies consuming food containing 0.25% aphoxide for 24 hours were completely sterilized. Feeding for 3 days on a diet containing 0.0025% aphoxide prevented or greatly reduced oviposition and none of the eggs hatched. Males fed for 3 days on 0.005% aphoxide were incapable of fertilizing normal fertile females. Longevity of adults was not affected in these tests. ENT-26398, 50106 and 50107, at 1% in the diet produced complete sterility but were only partly effective at 0.25%.

4. Horn Fly. In Texas horn flies were sterilized by feeding overnight on a bait containing 0.05% tepa but a concentration of 0.01% was ineffective. Topical applications of 0.1 ug/fly fully sterilized both sexes of flies.

In Oregon adult horn flies were sterilized by exposing the pupae to 5000 r.

5. Screw-worm. In Texas where studies with the screw-worm were conducted, 57 of 350 compounds screened as candidate chemosterilants caused sterility when administered as topical treatments or fed to adults. Some of the compounds sterilized by both methods of administration. Secondary tests showed that some of these compounds sterilized one or both sexes completely, while others induced only partial sterilization or were ineffective. Tests with tretamine applied topically showed that adult flies could be sterilized when they were 1, 3, and 5 days old with equal facility. Males sterilized with thiotepa survived as well as untreated flies and competed equally with untreated males in mating with females. However, the treated males were not as sexually competitive as untreated males. When a single dose of thiotepa or tretamine, adequate to sterilize either sex, was given in two half-doses 24 hours apart, survival of the flies was not improved and, further, a loss in sterilizing effectiveness occurred. Tretamine and thiotepa completely or partially sterilized screw-worms when puparia were immersed in solutions containing these compounds or were injected with them. With immersion, washing of puparia decreased the sterilizing effect, indicating that adults obtained some or most of the sterilizing dose as they emerged from the puparium. Aerosol treatment of screw-worm adults with tretamine resulted in almost complete sterility.

Preliminary laboratory experiments indicated that ENT-50450 was as effective as gamma radiation in sterilizing screw-worm flies, and was superior in its lack of toxic side effects. Females mated to males sterilized with ENT-50450 continued to lay infertile eggs after the initial deposition of eggs indicating that sperm in spermathecae of females did not recover fertility. Males sterilized with ENT-50450 remained sterile throughout their lifetime.



Investigations with screw-worm flies and aziridinyl-type chemosterilants resulted in the conclusion that the primary influence of aziridinyl compounds on the ovaries of flies 0-4 hours old is the inhibition of oogenesis, and of flies 1 day old, the induction of mutations. The effects of the aziridinyl compounds on the reproductive potential of female screw-worm flies were similar to those obtained with gamma radiation.

Studies were conducted to determine the absorption, metabolism and excretion of a  $P^{32}$ -labeled chemosterilant (MAPO) applied topically and in the diet of screw-worm flies. When applied topically the material was absorbed rather gradually. The absorbed material was metabolized to the extent of 42 and 58% in males and females, respectively, in 24 hours. The principal metabolic products were phosphoric acid and an unknown intermediate. Small amounts of five other intermediates were indicated but were not identified.

In the feeding tests, metepa was absorbed and distributed at about the same rates as in the topical tests. After 24 hours, the degree of metabolism in females was about the same as that in the topical test but, in the males, metabolism was 50% higher than in the topical tests. As in the feeding tests, the main metabolic products were phosphoric acid and an unknown intermediate. Five other intermediates were isolated but not identified. Approximately one-fifth of the applied dose of metepa was excreted.

Approximately 200 chemicals and other materials were screened as attractants for screw-worm flies. Of these, 10 were equal to or better than the standard liver bait and require further evaluation. Preliminary studies have indicated that mutant strains of screw-worm flies may respond differently from normal strains to attractants since black-mutant flies were not attracted to the standard liver bait.

6. House Flies. Research on the development of sterilization for the control or eradication of house flies has been continued in Florida and Oregon. In Florida 886 new chemicals were screened for sterilant activity. Of these some produced toxic effects; however, 90 caused complete or partial sterility in the treated house flies. Seven compounds were highly effective in sterilizing both sexes of house flies, but two of these were also toxic at the dosages tested. Metepa and tepa as residual deposits on glass sterilized house flies at dosages from 25 to 250 mg. per sq. ft., but 5-fluoroorotic acid was ineffective as a residual deposit at similar dosages. Apholate in the adult food sterilized house flies regardless of their age and the flies did not regain fertility. Motile sperm were present in the testes of chemosterilized males and transferred during copulation to the spermathecae of females throughout the life span of the male.

Tests were initiated in Florida to determine whether a dosage of chemosterilant too low to prevent hatching or adult emergence might by the accumulation of small genetic injuries eventually reduce or eliminate reproduction. With apholate, one colony showed reduction of oviposition in the 4th, 5th, 6th, and 7th generations and no individuals reached the pupal stage in the 7th



generation. With metepa, the 5th through 9th generation of another colony showed reduced oviposition and the 10th generation failed to oviposit.

Preliminary tests with apholate, tepa, and metepa, on house flies indicated that the probit of the percent sterility with house flies can be related to the log of the concentration.

Metepa and apholate shortened the life span of adult house flies considerably, but 90% or more of the male population survived the first 10 days, or that period of time in which mating activity is the greatest. Survival during the first 10 days was essentially the same in treated and non-treated house flies. Dipping house fly pupae in solutions of tepa, apholate, or metepa was toxic to most individuals, but a high degree of sterility occurred in individuals surviving the treatment.

Effective formulations of both dry baits and paint-on liquid baits have been developed.

A method has been devised for studying chromosomes of house flies. Using this technique, the effect of chemosterilants on spermatogenesis and oogenesis will be made.

Studies conducted in Oregon with an olfactometer and with simulated treated fly models (pseudo flies) demonstrated the presence in female house flies of a volatile chemical or chemicals which can influence the behavior of male flies. The behavior modification elicited was in the nature of attraction to a source of the pheromone, or an excitation of mating behavior patterns. The material which is benzene soluble and relatively stable was shown to be sex related, and appeared to be specific to the house fly since extracts of neither the face fly or stable fly affected the behavior of male house flies.

In Oregon tests were conducted to determine sterilizing effects of tepa and metepa on the little house fly (Fannia canicularis). All flies feeding on bait containing 0.1% tepa for 4 days and 0.5% tepa for 2 days succumbed in 3 and 5 days, respectively, and none oviposited before death. Flies feeding on 0.05% tepa for 3 days survived normally but did not oviposit. Metepa at 0.5% was highly toxic to flies. Flies feeding on baits containing 0.05 and 0.1% metepa survived and laid a few eggs but none hatched. Longevity of flies exposed for 2 hours on 100 mg. per sq. ft. residues of tepa was greatly reduced and all flies so treated were fully sterilized. Flies exposed on residues of 1 and 10 mg./sq. ft. laid a few eggs but none hatched. These tests indicate that the little house fly is much more easily sterilized than the house fly.

Over 50 inorganic and organic materials were tested as attractants for the little house fly. None showed significant attractancy.

In cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions, studies were continued in new facilities at Beltsville, Md., to



develop physical and mechanical methods of controlling house flies and other flies affecting cattle. Colonies of flies were established for conducting laboratory and outdoor cage studies. Studies to date have been concerned primarily with the attractiveness of various flies to different kinds and intensities of light. Black light ultraviolet radiation was attractive to both house flies and face flies during twilight periods. The use of fluorescent panels behind light sources increased their attractiveness.

7. Cattle Grubs. In Texas studies were made to determine the radiation dosage required to sterilize cattle grubs (Hypoderma lineatum). Complete sterilization was obtained by exposing pupae to 5000 r. A dosage of 2500 r sterilized the females but not the males.

8. Ticks. Extensive tests were conducted in Texas to determine the effects of different levels of radiation on different stages of the lone star tick. One series of tests with nymphs which had been engorged 2 weeks were exposed to different levels of radiation and the adults subsequently placed on hosts. Adults from nymphs exposed to 500 r or 1000 r engorged but produced no eggs. At a dose of 2500 r adults engorged normally and treated females mated with treated males did not oviposit. One of 8 treated females mated with normal males oviposited and some of the eggs hatched. When females and males treated with 5000 r were confined on hosts neither sex engorged. Similar results were obtained with untreated males and treated females, but when treated males and untreated females were used, 2 of 3 females engorged and oviposited. One of the two egg masses obtained was not viable and the other showed only a partial hatch.

Exposure of newly emerged adult ticks to 1000 r and 2500 r did not affect engorgement and complete sterility was indicated in crosses of treated males and females and in crosses of untreated males and treated females. Untreated females mated with treated males produced eggs but none hatched. One of the ticks exposed to 5000 r engorged but it did not oviposit. No ticks engorged after treatment with dosages of 7500 r or 10,000 r.

Some of adult ticks dipped in concentrations of 0.25 and 0.5% of apholate, tepa, metepa, or tretamine were killed but those that survived engorged and laid viable eggs. Dipping in 1% concentrations of these materials also failed to produce complete sterility but the maximum viability of eggs from females treated with apholate was only 0.25%.

#### F. Evaluation of Equipment for Insect Detection and Control

1. In Texas, in cooperation with the Agricultural Engineering Research Division, studies were undertaken to develop control mechanisms for automatic spraying devices that would be stable and dependable and which would not excite cattle. Step-on electric switches (switch-mats) installed on the ground underneath the spray nozzles were superior to the usual electric eye switches. The step-on switches provided quick and reliable operation of sprayers and required only limited maintenance. Cattle were not aware of



the imperceptible movement when they stepped on the mat and activated the sprayer. Most of the commercially-available automatic sprayers have mechanical defects and several adaptations were tried as improvements. One adaptation of the ring-type fitted with 7 nozzles proved superior to the commercial types.

At Beltsville, Md., in cooperation with the Agricultural Engineering and Animal Husbandry Research Division, work was initiated to develop test equipment and techniques of operation to evaluate the attractiveness of visible and ultraviolet radiation to house flies or other flies affecting cattle. This research has not yet reached a point of profitable summation.

#### G. Insect Vectors of Diseases

1. Anaplasmosis. Studies were continued in Texas, Mississippi, and Oregon to correlate the presence and abundance of insects and ticks with the incidence of anaplasmosis in herds of cattle. These studies were conducted in cooperation with the Animal Disease and Parasite Research Division and veterinarians of the various State experiment stations. In Texas, monthly surveys were continued to determine the abundance and identity of external parasites on infected (anaplasmosis) and clean herds of cattle. Lone star ticks were abundant on cattle during May, June, and July, 1962, but declined rapidly in August and partially disappeared by September. During this same time, ear tick populations were high except for August. Horn flies became numerous early in May but periodical spraying of cattle minimized populations the remainder of the season. The winter tick and black-legged ticks appeared in November. The winter tick was abundant by December, but black-legged tick populations remained low. Moderate populations of the ear tick were present from October through December. Small numbers of lone star and black-legged ticks and large numbers of ear and winter ticks were present on cattle in January, 1963. In February and March, 1963, populations of the lone star tick increased, while the winter and black-legged ticks virtually disappeared. Ear tick populations remained high.

Prophylactic treatment with aureomycin minimized transmission of anaplasmosis and prevented acute cases. All of the 10 animals in the control herd contracted the disease and 3 of them had acute cases. At the low level (2 mg.) the maximum number of negative animals occurred after 3 months of treatment, whereas at the high level the maximum number occurred 3 months after treatment was terminated. In time, most of the negatives reverted to positives. The period of greatest transmission of the disease coincided with that in which the horse flies, Tabanus lineola and T. vittiger schwardti, and the mosquito, Psorophora confinnis, were most abundant.

In Maryland, hereditary transmission experiments and histopathological studies with the vectors of anaplasmosis of cattle were continued in cooperation with the Animal Disease and Parasite Research Division. Two series of transmission trials with the vector, Dermacentor andersoni, were conducted

In one, the  $F_1$  larval and adult progeny of female ticks fed on an animal in the acute stages of anaplasmosis failed to transmit anaplasmosis to test calves. In tests with larval progeny, challenge of the animals with the disease proved the animals to be susceptible to the disease. Proof of the susceptibility of animals used with adult progeny has not been completed. In another series of trials, unmated and mated males fed on an animal with acute anaplasmosis were studied for survival under hibernating conditions (4-5°C. and 30-50% relative humidity with a photoperiod of 7 hours) and their ability to transmit anaplasmosis. Mated males survived only about 4 months in the hibernating environment and transmission trials could not be run. Eleven of 49 unmated males survived the 8-month-hibernating period, and 6 of these survived for transmission trials 3 months after the hibernating period. None of the survivors transmitted anaplasmosis to test cattle; however, not all of the animals have been proved susceptible by challenge at this time. In similar studies previously reported, transmission by infected males was demonstrated.

In Maryland, histopathological studies on Anaplasma-infected and non-infected Dermacentor andersoni were continued. Using immunofluorescence methods, anaplasmata were found in gut and excreta smear preparations from ticks fed on infected animals. Brightfield and electron microscopic examinations revealed anaplasmata in excreta. Electron microscopic studies showed structures believed to be A. marginale in undigested erythrocytes. However, anaplasmata were not found in ultrathin section of the salivary glands and reproductive organs.



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EQUIPMENT AND BUILDINGS USED IN PRODUCING DAIRY PRODUCTS  
Agricultural Engineering Research Division, ARS

Problem. Economic conditions are causing farmers to step-up their efforts to reduce production costs and improve quality by reducing labor and modifying environment in livestock production. Labor is an important element in production costs. How to make better use of equipment and to adapt existing buildings and other facilities for more efficient production as herds are increased in size and farms consolidated are major considerations. Cost of replacement or major improvement of existing buildings that are not suited to modern production methods are serious obstacles. Principles, examples, and techniques for planning more efficient operations are needed both by farmers doing their own engineering and by those on whom farmers depend for advice.

USDA PROGRAM

This is a continuing program involving engineers and architects conducting basic laboratory investigations, application of laboratory results to a production basis, and development of typical plans for livestock structures. The work is in cooperation with the AH, ADP, and ENT Divisions of ARS, USDA, and State Agricultural Experiment Stations, and contributes to Cooperative Regional Projects NC-23, "Farm Structures to Meet Environmental Requirements of Dairy Cattle, Swine, and Poultry", S-49, "Genetic Methods of Improving Dairy Cattle for the South", and NE-8, "Essentials of Poultry Housing for the Northeast." Plan development work is cooperative with all the State Agricultural Experiment Stations and Extension Services.

Dairy cattle environmental and bio-engineering studies are conducted in a climatic laboratory at Columbia, Mo., in cooperation with the Dairy Husbandry and Agricultural Engineering Departments of the Missouri Station. AH, ARS, serves in an advisory capacity. Field studies in a hot, humid region are conducted at Tifton, Ga., with the Georgia Coastal Plain Experiment Station and AH, ARS, cooperating. The influences of building arrangement, equipment, and chore routines on the amount and drudgery of dairy chores and means of improving these factors are studied in cooperation with the California Agricultural Experiment Station. Typical plans for dairy structures are developed at Beltsville as part of the Cooperative Farm Building Plan Exchange. The Federal part of the work described in this paragraph involves 2.2 professional man-years.

At the Washington State Experiment Station equipment and control for automatic feeding of livestock is under development and work on performance characteristics of upright-silo unloaders is in cooperation with the Minnesota State Experiment Station. This work is applicable to both beef and dairy feeding operations. The Federal effort amounts to about 2.0 professional man-years.



Other work is devoted to reducing pesticide residues, conducted at Kerrville, Texas; physical methods of fly control and methods of cooling milk on farms, conducted at Beltsville, Md. The work on residues involves 2.2 professional man-years; the engineering program devoted to electrical and physical insect control involves 6.4 man-years of which part is applicable to dairy; and the milk cooling work involves 0.3 man-years. The work is cooperative with ENT, AH, and EU, where appropriate.

## REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

### A. Dairy Cattle Engineering

1. Increasing efficiency of operations. At Davis, Calif., work was continued in cooperation with the Experiment Station on a study of dairy layouts having herringbone milking rooms. As herringbone milking rooms were constructed and became available for study with the large-scale dairy installations, they have been studied to determine their effectiveness as a means of reducing the labor requirements in these large installations. As a result of this work, plans for pie-shape layouts with herringbone milking rooms have been developed.

Also in cooperation with the Experiment Station at Davis, work has continued on analysis of a large volume of time and travel data that have been recorded on the large-scale dairy farmsteads. This material has been used as the basis for several publications and is now being worked up into a consolidated form to present the principles of designing an efficient dairy farmstead layout. Some of these principles have been incorporated into the design of a trial pie-shaped layout which has been constructed in the past year and which will be studied as a means of corroborating the tentative conclusions.

2. Bio-engineering studies. Basic fundamental studies on the relationships between environment and various dairy animal health and production factors were continued in the psychroenergetic laboratory, and related facilities at Columbia, Mo., in cooperation with the Missouri Agricultural Experiment Station. A 9-week test was made on 6 lactating Holstein cows to test their ability to acclimate to 85° F. temperature and 50% relative humidity (RH). Measurements at 85° F. were compared with those at 65° F. and 50% RH immediately preceding and following the 85° F. exposure.

Individuals varied in initial and acclimation responses to heat exposure with respect to their milk production, feed and water consumption, loss of body weight, and rise in body temperature. Losses in production were significant ( $P < .01$ ) during the 9-week exposure in 4 of 6 cows. Three of the 6 were much more heat-sensitive than the other three. The heat-sensitive group lost 407 pounds in 9 weeks; the others lost only 166 pounds. Production decreased in all cows during the first 2 weeks at 85° F. A significant ( $P < .05$ ) recovery occurred in the less heat-sensitive group, but not



in the other group, during the last 7 weeks at 85° F. Milk production in the three highest producers dropped 449 pounds but only 151 pounds in the three lowest producers. Feed and water showed acclimation trends but no significant group differences.

Cooling inspired air to about 60° F. and 50% RH allowed recovery of milk production for animals previously exposed to 85° F. and 50% RH, and with these conditions continuing to surround the animals' bodies at the time of the test. This recovery was evident in 3 of the 4 cows undergoing preliminary testing. Insulated units installed so as to surround the head of a stanchioned cow permitted control of the conditions of the inspired air. Actual amounts of recovery will not be known until the persistency of lactation can be computed. Cows showing a 1° F., or higher, rise in rectal temperature in response to the 85° F. room condition (following a 65° F. room condition) showed a decrease of about 1° F. when inspiring cooled air in the hot environment.

Physiological and genetic evaluation of dairy cattle also was continued at Missouri. Standardized environmental conditions were used to physiologically evaluate the 12 cows entering the climatic laboratory for tests during the year. This is a continuation of the long-range study reported last year. Responses of the animals were again quite varied. At a condition of 88° F. and 40% RH, maximum differences among individuals were 4.2° F. in rectal temperature, 40 respirations per minute, and 20 heart beats per minute. These differences are similar to those reported among individual cows last year.

Heat sensitivity of lactating cows was studied in tests initiated at Missouri to evaluate the potential of using a standard hot environment for determining the heat sensitivity. A single-cow hot box was developed to provide the standard environment of 110° F. with no solar load. The primary measure was the length of time required to cause an arbitrarily selected rectal temperature rise of 2° F., thus providing a line of given slope for each cow tested. Theoretically, this slope will be a measure of heat sensitivity. Tests of repeatability of slope values were made on 2 cows and, in general, the slopes were quite consistent for each cow. Thirty-six randomly selected lactating cows were later subjected to the standard environment. These cows apparently fall into 2 groups, one group having a low rate of rectal temperature rise (heat tolerant) and the other having a higher rate of rise (heat sensitive).

Environmental measurements on a Missouri dairy farm were started during January 1963. In conjunction with a nutrition study involving 2 lots of 20 lactating Guernsey cows each, a weather station was set up to record dry bulb and black globe thermometer temperatures and air velocity in the outside lot areas. Hygrothermographs were placed to record air temperature and relative humidity in the loose housing area within the open-front shelter. This study is still in progress.



Production methods for cooling dairy cattle were studied at Tifton, Ga., in cooperation with the Georgia Station. A continuing study was made of the comparative value of shade versus shade plus fans plus water sprays on lactating Jersey cows. Two groups of 9 cows each were held in drylot at all times during a 63-day test except for the milking time. The only difference between the two groups was the shelter system. There was no difference in rate of decline of milk production between the two treatments. However, mastitis was a serious problem during the test and may have influenced the results. Activity observation at 15-minute intervals on 10 days showed that during daytime the cows in both treatments used the shades the same percent of time and spent essentially the same time eating. Animals in both treatments spent one hour at the feed trough in daytime and 2-1/2 hours at night.

3. Plan development. At Beltsville, Md., a plan was developed for a combination hay storage and feeding shed suitable for dairy and beef cattle use throughout the South (and to some extent in other regions). A plan was also developed for a cattle feeding shelter suitable for the South and West and having covered feed bunks with poles in the center so that an auger can work between the poles or a self-unloading wagon can empty directly into the bunk. These plans were developed for the Cooperative Farm Building Plan Exchange.

#### B. Dairy Cattle Equipment

In Minnesota the performance of electric motors for silo unloaders is being determined in cooperation with the University of Minnesota Agricultural Engineering Department. Two makes of standard late model unloaders were installed in two concrete stave silos filled with wet corn silage. The moisture contents of the silages ran 81% and 83% on a wet basis. The silages on top of the silos were slightly dried out and the unloaders were able to unload the material. At a depth of 5 feet from the top, both units ceased to deliver any silage. Power demands by this time had doubled. The speeds of the blowers in both units were reduced by 30% and unloading again was possible. The speed reduction and a slight blower housing modification by the manufacturer made possible complete unloading of one silo with one make. In the other unit impeller blades of a different design were installed in addition to the speed reduction to enable complete unloading of the silo.

Performance tests of specially designed capacitor motors would indicate ability to operate unloaders if adequate voltages are maintained. One of the repulsion induction motors now in common use did not meet the nameplate ratings for duty cycle. Use of a silo unloader to feed an Experiment Station beef breeding herd reduced labor costs by 50% when the silage was frozen and by 70% when it was not frozen.



In Washington an automatic horizontal or trench silo unloader is being developed in cooperation with the Washington State University Agricultural Engineering Department. The cutter unit developed in 1962 was tested with two 32-inch long, 16-inch diameter augers. These larger augers were more effective than the previous 12-inch diameter augers, removing more than 600 lb./min. of peavine silage. With an input of 2 horsepower the cutter will deliver more than 400 pounds of peavine silage per minute. The major work now in progress is the incorporation of the cutter with suitable conveyors to remove the silage from the silo. This has resulted in the development of an experimental unloader. The present unloader design requires 7-3/4 horsepower. When operational difficulties have been overcome, automatic controls will be added.

#### C. Reducing Pesticide Residues in Animal Products

Field tests of experimental automatic walk-through sprayers for controlling flies on cattle, conducted at Kerrville, Texas, in cooperation with the Entomology Research Division, indicated that they controlled horn flies on cattle as well as did power sprayers used in pens, and used 37% less insecticide. The insecticide used was one of the two that can be sprayed directly on lactating cattle without leaving residues in the milk. The sprayers appeared to be suitable for herds that must come to specific watering spots in their dairy routine or that must be milked (in the case of dairy cattle). Operation of the sprayers during the field tests indicated that reliability without supervision and speed of operation are factors which must be optimized for the most efficient use.

In developing and evaluating equipment and procedures for reducing chemical hazards associated with the control of livestock insects a measure of the amount and location of the spray applied to each animal is very important. At Kerrville, Texas, methods of quickly and accurately measuring the spray retention have been evaluated and a number of them discarded. The use of a fluorescent dye tracing technique is presently being studied as a means of measuring both distribution and retention. Preliminary trials indicate that only 38% of a one-gallon spray of clear water will remain on a mature Hereford cow when the application is made with a power spray.

#### D. Physical Methods of Fly Control for Dairy Farms

Investigations of physical methods for controlling flies in and around dairy barns are being conducted at Beltsville, Md., in cooperation with the Animal Husbandry and Entomology Research Divisions, ARS. Construction of a new laboratory building for this research was completed in November 1962 at Beltsville, Md. Preliminary studies conducted in temporary facilities at Orlando, Fla., and at Beltsville indicated that blacklight ultraviolet radiation is attractive to both house flies and face flies during twilight periods. Use of fluorescent panels behind the light sources appeared to increase attractiveness.



## E. Methods of Cooling Milk on Farms

Preliminary studies were initiated to determine the relation of milk quality to various cooling procedures for on-farm cooling of milk. Initial efforts concern assessments of facilities and equipment by test to provide performance information on installed cooling apparatus. Both ice bank and direct expansion equipment have been calibrated to produce cooling rates of 115, 170, and 340 Btu/hr./gal. Cooling rates are controlled on the ice bank tank by intermittent operation of circulation pump and on the direct expansion tank by modulation of refrigerant flow. The tanks and equipment have been instrumented to record equipment performance factors such as temperatures, refrigerant pressure, electrical energy, and operating time. This work is cooperative with the Animal Husbandry Research Division and the Eastern Utilization Research and Development Division.

### PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

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Johnson, J. C., and Givens, R. L. 1962. Summary of studies on the effects of shade, fans and sprinklers on summer milk production. Presented at Annual Dairy Field Day, Gainesville, Florida.

Watering trough (continuous flow) for cattle. 1962. (Exchange Plan No. 5909). USDA Misc. Pub. No. 898, April. (Note: Also listed under B, below).

The following cooperator's publications are the result of cooperative work and report related non-engineering phases of the research:

Kibler, H. H. 1962. Energy metabolism and related thermo-regulatory reactions to thermal stress in the 50° and 80° acclimated dairy heifers. Missouri Agr. Exp. Sta. Res. Bul. LXI.

### Cattle Feeding Equipment

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## II. NUTRITION, CONSUMER AND INDUSTRIAL USE RESEARCH

### NUTRITION AND CONSUMER USE RESEARCH

Consumer and Food Economics Research Division, ARS  
Human Nutrition Research Division, ARS

Problem. The assortment and characteristics of foods available to consumers are constantly changing with the adoption of new production, processing, and marketing practices. Constantly changing also, as nutrition science advances, is our understanding of the nutritional needs of man and the manner in which these needs can best be met by food. To help carry out the Department's responsibility to advise on the quantity and variety of foods that will assure maximum benefit and satisfaction to consumers, continuous research is essential on the nutritional requirements of persons of all age groups, and on the nutrient and other inherent values of foods and how to conserve or enhance these values in household preparation and processing. Periodic examinations of the kinds and amounts of foods consumed by different population groups and individuals also are essential for evaluation of the nutritional adequacy of diets and to give the guidance needed for effective nutrition education. Such information provides assistance needed in market analyses for different commodities and in the development and evaluation of agricultural policies relating to food production, distribution, and use.

### USDA PROGRAM

The Department has a continuing program of research concerned with (1) nutritive and other consumer values of raw and processed foods as measured by chemical or physical means and by biologic response; (2) effects of household practices upon the nutritive values and inherent qualities of foods, and the development of principles and improved procedures for household food preparation, care and preservation; (3) surveys of kinds, amounts, and costs of foods consumed by different population groups and the nutritional appraisal of diets and food supplies; and (4) development of guidance materials for nutrition programs.

The research is carried out by two divisions of the Agricultural Research Service--the Human Nutrition and the Consumer and Food Economics Research Divisions. Most of the work is done in Hyattsville, Maryland, and at Beltsville, Maryland; some is done under cooperative or contract arrangements with State Experiment Stations, universities, medical schools, and industry. The total Federal scientific effort devoted to research in these areas totals 66.3 man-years. It is estimated that approximately 6.5 man-years is concerned with studies related to dairy and dairy products.

Human metabolic studies and the related exploratory and confirmatory studies with experimental animals and microorganisms concerned with defining human requirements for nutrients and foods are not reported on a commodity basis,



though some of the work is applicable to this report. This basic nutrition research represents a total Federal effort of 23.4 professional man-years and is described in detail in the report of the Human Nutrition Research Division. Certain aspects of this research related to dairy products are considered briefly in this report.

## REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

### A. Nutrient Values of Dairy Products

1. Tables of food composition. The 1963 revision of Agricultural Handbook No. 8, "Composition of Foods...Raw, Processed, Prepared," was completed and carried through to the galley proof phase. Composition data for about 100 items of milk and predominantly milk products are included. In addition to cows' milk at both 3.5 and 3.9 percent fat, data are included for goat, reindeer, and human milk. The list of cheeses has been expanded to 16 and includes 3 processed cheeses and 2 types of cottage cheese. A number of baby foods and soups which contain varying proportions of milk products are included as well as several mixed dishes containing milk products, such as cheese fondue and various beverages.

Data in the popular publication, "Nutritive Value of Foods," Home and Garden Bulletin No. 72, have been revised to agree on a weight basis with nutritive values in Handbook No. 8. The revised edition will provide nutritive values of household measures of 512 commonly used foods. Another popular publication, "Conserving Nutritive Value of Foods," Home and Garden Bulletin No. 90, is in press.

2. Proteins and amino acids. A manuscript was published describing a method developed for assay of alanine using Leuconostoc citrovorum 8081 and providing analyses of 48 proteins and foods including milk and milk proteins.

3. Biological value. The nutritional value of various components of milk are being investigated with rats by feeding diets containing milk protein and fat as butter oil or corn oil and carbohydrate as lactose or cornstarch. Other rats are being fed dried skim milk and butter oil. At 400 days of age, the effects on body composition and structure will be determined by necropsy, assessment of aorta damage, proximate analyses on carcass and liver and analysis of liver and serum for content of cholesterol and other lipids.

Lipid biosynthesis is being studied as a possible criterion for assessing the nutritional value of foods. Rats developed acute deficiency symptoms when fed a cholesterol-free diet plus an inhibitor of cholesterol biosynthesis. The content of total sterols in their carcasses and tissues was about the same as for control animals fed a cholesterol-free diet. Analysis of the major sterols by Entomology Research Division showed 75 percent of the total sterols to be desmosterol and less than 20 percent to be cholesterol in the carcasses of the inhibitor-fed rats; in the carcasses of



control rats 95 percent of the total sterols was found to be cholesterol. A manuscript presenting these findings has been accepted for publication. In other phases of this research, lipid biosynthesis in relation to age and diet is being investigated.

#### B. Properties Related to Quality and Consumer Use of Dairy Products

Shortening properties of fats. Investigation of the shortening properties of five kinds of fat--corn oil margarine, hydrogenated vegetable fat, hydrogenated vegetable and animal fat, regular margarine, and butter at different levels of added fat, liquid, and sugar in white cakes is in progress. Sensory, physical, and chemical measurements are being used to determine the influence of the proportion and kind of fat on the quality of the baked product. A report on levels of fats and oils in pastry and biscuits was accepted for publication in Cereal Chemistry.

#### C. Nutrient Functions

Lipids. A better understanding of specific relations between diet, health and longevity has resulted from long-term investigations with laboratory animals fed 29 different experimental diets, including ones containing skim milk or milk proteins and butter fat. Both excessive food intake and relationship or balance of nutrients in the diet are implicated in the adverse effects that occurred throughout the lifespan of laboratory animals. The studies indicate that genetic strain affects the response to the different diets and thus emphasize the importance of recognizing inherited characteristics in evaluating response to diets. Survival varied even with diets of similar fat and protein content. Differences in serum cholesterol levels of animals showed no relationship to kind or level of fat nor to level of dietary cholesterol.

#### D. Food Consumption and Diet Appraisal

1. Food consumption and dietary levels. A report of the findings of the food consumption survey of beneficiaries of Old Age and Survivors Insurance made in Rochester, New York in the spring of 1957 has been completed. The survey included 283 1- or 2-person households. During the survey week, food brought into the kitchens of these households averaged about the following amounts per person: 4 quarts of whole milk or its equivalent in milk products; 4 pounds of meat, poultry, fish; 1/2 dozen eggs; 10 pounds of vegetables and fruits; 2 pounds of grain products (in terms of flour); 1 pound of sugars and sweets; and 3/4 pound of fats and oils. The total money value of all food per person was \$8.12. Nutrients from this food more than met the National Research Council's recommended allowance for the average person. However, less than half (44 percent) of the households had diets which met in full the recommended amounts for all nine nutrients (good diets). Nearly three-fourths of the households had diets that met



two-thirds of the recommendations for all nutrients (good and fair diets). The nutrients which fell below the recommended allowances most often were thiamine and calcium.

The series of food surveys conducted in low-income areas to aid in the study of the effects of food distribution programs on diets of families has been extended to include a survey carried out in Choctaw County, Oklahoma and in Pensacola, Florida. These were conducted cooperatively with the Marketing Economics Division, Economic Research Service as were similar surveys reported previously.

A food consumption survey was carried out in the District of Columbia that will provide information on the diets of households and of individuals. The study was undertaken primarily as a pilot survey in developing procedures for the next Nationwide survey proposed in the Department's long-range program.

An analytical study of consumer response to the relative price of store versus delivered milk was supported in part by the Consumer and Food Economics Research Division, and reviewed by staff members. The variables introduced were the relative quantities of milk purchased in stores or delivered, the relative prices of the store and delivered milk, average income, household size, and purchases of bread and coffee (as proxy variables indicating composition of the household). The findings suggest that consumers are more highly responsive to the relative prices of store and delivered milk than has been assumed.

The nutrient content of the per capita food supply is calculated and published each year, using data on estimated quantities of foods consumed (retail-weight basis) as developed by the Economic Research Service. This series, with estimates extending back to 1909, is the only source of data on year-to-year changes in the nutrient content of the U. S. per capita food consumption. A comparison of the dietary fat provided by the individual foods within the fats and oils group in 1930 and 1962 revealed important changes. The proportion of dietary fat furnished by butter and lard in the fats and oils group decreased markedly--butter from 25 to 10 percent, and lard from 22 to 14 percent. These declines were compensated for by larger consumption of margarine, shortening and salad and cooking oils.

2. Food management practices. The results from three small studies based on records kept by the homemaker on the kind, amount, and nutritive value of foods used and discarded in households have been prepared as a journal article. In terms of total calories available for consumption, discarded edible food averaged 7 percent in St. Paul, Minnesota; 8 percent in DeKalb County, Missouri; and 10 percent in Los Angeles, California. A study using "recall questions," instead of records, with a random sample of 300 households in Minneapolis-St. Paul in the winter of 1960 is currently being processed.



A report on household practices in handling and storing commercially frozen foods, based on surveys in two cities has been published. Survey findings indicate that household practices alone would not cause serious quality deterioration of frozen foods.

A new study has been initiated (under contract) of the management practices of urban and farm home freezer owners in Fort Wayne, Indiana and a nearby rural area. The survey is designed to obtain information on such actual management practices of home freezer owners as the kinds, amounts, sources, prices, and rate of turnover of foods frozen and stored in the home.

3. Development of food budgets and other basic data for food and nutrition programs. The ongoing program of interpretation and application of nutrition research findings to practical problems for use by nutritionists, teachers, health workers, and other leaders concerned with nutrition education or nutrition policies has involved the preparation or review of articles and publications, talks, television interviews, and participation in various conferences and committees.

With the publication of the report "Family Food Plans and Food Costs" the technical work on the development of the Department's current low-cost, moderate-cost and liberal food plans was completed. The continuing phases of the work on individual and household food budgets consists in the regular pricing of the food plans for publication in Family Economics Review, and in dissemination of information concerning them through such popular publications as "Family Food Budgeting for Good Meals and Good Nutrition," through filmstrips ("Food for the Young Couple"), and through correspondence, talks and committees (such as the Advisory Committee to the Bureau of Labor Statistics on their City Workers' Standard Budget).

Progress on the revision of Handbook No. 16, "Planning Food for Institutions" has focused primarily on the food purchasing guide section. Publications in preparation that are designed for the use of teachers, extension workers and other leaders are (1) a semi-popular publication on nutrition in the series Facts for Nutrition Programs; (2) a report on fat and related components in U.S. diets; and (3) a study of the relative economy of foods.

Nutrition Committee News, a bimonthly periodical prepared for members of State nutrition committees and other workers in nutrition education provides one channel for disseminating pertinent information and for reporting nutrition education activities. Examples of subjects of current interest covered during the report period are: "Nutrition Aspects of Selected Studies of Cardiovascular Diseases--Implications for Nutrition Education," "Planning Nutrition Programs for Elementary School Teachers," and "Food Guides--A Teaching Tool in Nutrition Education."



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Nutrient Values

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Food Consumption and Diet Appraisal

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Development of Food Budgets and Other Basic Data for Food and Nutrition Programs

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DAIRY PRODUCTS - CHEMICAL, PHYSICAL AND BACTERIOLOGICAL CHARACTERISTICS;  
DEVELOPMENT OF NEW AND IMPROVED PRODUCTS AND PROCESSING METHODS  
Eastern Utilization Research and Development Division, ARS

Problem. Dairying is one of the largest segments of American Agriculture: dairy products represent about 13 percent of all farm cash receipts; milk production requires 140 billion feed units annually; milk is a highly nutritious food. It is clear from these facts that research which succeeds in increasing the consumption of milk will have far-ranging effects in raising nutritional levels, in increasing farmers' income, and in increasing consumption of feeds. There is opportunity to increase milk consumption, for per capita consumption is currently at its lowest point in over 30 years at 610 pounds, whole milk equivalent. Current consumption in the U. S. is well below that of several foreign nations, including New Zealand, Canada, Australia, Sweden, Norway and the United Kingdom, all using more than 800 pounds per capita.

Increased consumption can result from improved quality of manufactured dairy products, from cost reductions based on improved processing technology, from the development of new products, or from any combination of these. The development of new and improved processes and products is the objective of utilization research on dairy products.

Both basic and applied research in this field are needed; applied research is the direct antecedent to the development of new products and processes, and basic research provides the information which permits applied research to proceed most effectively.

Increased emphasis on basic research has been advocated by the Dairy Research and Marketing Advisory Committee, and the National Agricultural Research Advisory Committee. Basic research is considered primarily the responsibility of public agencies which disseminate their findings for use by all.

One aspect of the problem posed by dairy products is the great need for fundamental information on the complex biophysical-chemical system which each dairy product is. The development of new products and new processing technology through applied research represents the exploitation of fundamental information. Such exploitation and development cannot continue indefinitely; the supply of fundamental information must be maintained and enlarged, and this is the purpose of basic research. The complexity of milk makes necessary the employment of several scientific disciplines in basic research on this commodity. These disciplines undertake investigations needed to identify and measure the amounts of individual chemical components present; the molecular structure of these components; how the molecules react; and the forces which determine the course of the reactions. These studies should be intensified. Other needed investigations include study of the mechanism of the synthesis of milk; the properties of milk fat; and

the factors responsible for the flavor of dairy products and the changes in flavor which occur during processing and storage.

There is also need for a vigorous and sustained program of applied research which is aimed to increase consumption of dairy products. Such a research program could stimulate consumption by development of products with increased palatability, convenience, or extended shelf life. Another opportunity is the possibility of developing new and improved processing technology which will reduce costs. Because the price elasticity of milk and milk products is greater than that of most food crops, cost reduction is an attractive avenue for increasing consumption.

Still another opportunity is the development of new milk products of low fat content, for example, a low-fat Cheddar cheese. Such a development could alleviate problems posed by current controversy over the effect of animal fats in the diet.

Increased consumption, however achieved, should have a powerful upward effect on feed consumption. It is estimated that milk production requires 140 billion feed units annually (a feed unit is the feed value equivalent to a pound of shelled corn). Hence a 1 percent increase in production would require feed equivalent to 25 million bushels of corn--the production of some 500,000 acres. If the feed were supplied by cropland pasture, more than a million acres would be needed.

It is thus manifest that utilization research leading to product and process development can provide a powerful stimulus to American agriculture.

#### USDA PROGRAM

The Department has a continuing long-term program involving chemists, biochemists, microbiologists, food technologists, and engineers, engaged in basic research on the composition and properties of milk, and in applied research directed to the development of new and improved dairy products and processing technology.

The Department's research facilities are located in Wyndmoor, Pennsylvania, Washington, D. C. and Beltsville, Maryland. The Federal scientific effort devoted to research in this area totals 73.7 professional man-years. The effort is distributed as follows:

(a) Work on improved concentrated milks involves 11.0 p.m.y. at Washington. Contract research is under way at the University of Illinois, Urbana on possible flavor improvements in concentrated milk; 0.3 p.m.y. is involved. Contract research at Oregon State University, Corvallis deals with methodology for evaluating concentrated milks; 0.4 p.m.y. is involved. Under a research grant to the Ohio State University Research Foundation, Columbus, research is under way (0.8 p.m.y.) on alteration of the inorganic colloidal calcium phosphate complex of milk, with the objective of improving the



stability of evaporated milk. In addition, research sponsored by the Department under a P.L. 480 grant is going forward at (1) Freddo Experiment Station, Milan, Italy on new food products from concentrated milk and fruit juices suited to European tastes; (2) National Institute of Agronomic Research, Paris, France on processing; (3) National Institute for Research in Dairying, Reading, England on the isolation and characterization of selected enzymes of milk to obtain fundamental information useful in improving the quality of dairy products; and (4) Frio Experimental Center, Madrid, Spain on factors responsible for protein destabilization during storage of frozen milk, for use in devising an improved frozen milk concentrate.

(b) Research on dry whole milk and other dried milk products involves 12.0 p.m.y. at Washington and Wyndmoor. The program includes fundamental and applied research directed toward developing technically and economically feasible methods for producing dried whole milk with instant dispersibility and with flavor which will remain stable during six months storage at room temperature and also improved means for drying nonfat milk, whey, ice cream mixes, buttermilk, etc. A research contract with the University of Wisconsin, Madison, provides for a study (10.3 p.m.y.) of the effect of nonfat dry milk on the biochemistry of bread yeast fermentation.

(c) Work on improved butter involves 2.0 p.m.y. at Washington.

(d) Research on improved ripened cheeses involves 3.0 p.m.y. at Washington and Beltsville. Contract research on the chemistry of cheddar cheese flavor is being conducted at the Ohio Agricultural Experiment Station, Columbus; 0.4 p.m.y. is involved.

In addition, research sponsored by the Department under P.L. 480 grants is going forward at: (1) Institute of Biochemistry, Turku, Finland on growth-promoting factors for lactic acid bacteria in cheese making; (2) Kaira District Cooperative Milk Producers Union, Ltd., Anand, India on addition of nonfat dry milk solids to buffalo milk in the manufacture of hard cheese; (3) Institute of Dairy Industry, Warsaw, Poland on increasing the vitamin B content of cheese; and (4) National Dairy Research Institute, Karnal, Punjab, India on milk coagulating enzymes of microbial origin, for cheese manufacture. This Institute also has a grant for the isolation and fractionation of components in the proteose-peptone fraction of milk. Additional research under P.L. 480 goes forward at: (5) National Institute of Agronomic Research, Paris, France on the activity of rennin toward individual components of casein; (6) National Institute for Research in Dairying, Reading, England on the application of partition chromatography and allied techniques to the differentiation of microorganisms important in dairy products; and (7) National Institute of Technology, Rio de Janeiro, Brazil on proteolytic enzymes.

(e) Work on flavor components and flavor stability of milk and milk products involves 5.9 p.m.y. at Washington. In addition, research sponsored by

the Department under P.L. 480 grants is going forward at: (1) Biochemical Institute, Helsinki, Finland on the transmission of flavor components and other biologically-active compounds of feed of dairy cattle to the milk and milk products; and (2) National Dairy Research Institute, Karnal, Punjab, India on the effect of the sulfur compounds in milk and milk products on flavor and oxidative stability.

(f) Work on the isolation, structure and properties of milk proteins, and the interactions of milk components involves 20.5 p.m.y. at Wyndmoor, in pioneering studies of milk proteins and investigations of the reactions of proteins and polypeptides with each other and with other milk components. Cooperation is maintained with the Animal Husbandry Research Division, ARS.

(g) Research on the identification and removal of radioactive nuclides from milk involves 8.0 p.m.y. at Beltsville. This is a cooperative program with the Atomic Energy Commission and the U. S. Public Health Service which provides 2.0 p.m.y. additional. The objective is the development of effective ways for removing cationic radionuclides (Sr-90, Cs-137, and Ba-140). A research contract with the Producers Creamery Company, Springfield, Missouri, is concerned with the development of systems for a commercial scale process for removal of radioactive contamination from fluid milk. The contract (7.3 p.m.y.) is supported equally by the Eastern Division and the U. S. Public Health Service.

(h) Research on the control of spoilage organisms involves 1.0 p.m.y. at Washington in basic studies of the formation, germination and heat resistance of bacterial spores.

(i) Work on genetics and milk composition involves 0.5 p.m.y. at Wyndmoor in a study of genetically-caused variation in the composition (and hence the interactions) of selected milk proteins. This work is cooperative with the Animal Husbandry Research Division, ARS. In addition, work sponsored by the Department under a P.L. 480 grant goes forward at the Research Center on Macromolecules, Strasbourg, France, on the structure of nucleic acids.

(j) Pioneering research on the allergens of milk and other agricultural products involves 4.0 p.m.y. at Washington.

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

##### A. Improved concentrated milks.

Recent progress further delineates the range of variations in milk composition and processing conditions that can be used for the preparation of stable HT-ST sterile milk concentrates by use of chemical additives. This work represents the first step towards the production of sterile fluid whole milk concentrates which have satisfactory storage life and will reconstitute to a milk of beverage quality. Experiments indicated that 4:1 sterile skim milk concentrates can be prepared with satisfactory resistance towards gelation,



sediment deposition, lactose crystallization and flavor deterioration. Sterile 2:1 and 3:1 concentrates of "2-10" milks were prepared with storage lives exceeding six months. Sterile high-fat 3:1 concentrates were also successfully prepared. Experiments showed conclusively that under a variety of conditions, substitution of laminar for turbulent flow during the holding period, and of evaporative for forced convective cooling served to prevent undue coagulation during HT-ST processing, and to reduce sediment deposition during storage by 200-300 percent. Hexametaphosphate in concentrates held at 70°F. for two months was largely degraded to pyrophosphate. Trace amounts of pyrophosphate were found in fresh milk and in control concentrates. Such results bear favorably on the question of the suitability of polyphosphates as food additives.

In fundamental research to further elucidate the reasons for gelation and its prevention, it was found that barium and strontium ions effected a marked increase in the storage life of milk concentrates. 0.15% cesium added to milk concentrates brought about a decrease in storage life, magnesium a slight increase. Barium was unique in that its presence did not impair heat stability. Fluoride ions behaved in a manner reminiscent of polyphosphate ions, but were much less effective. Between pH 6.0 and 6.5, the viscosity and storage life of sterilized polyphosphate-containing concentrates were independent of pH. Wide latitude is indicated in variations in composition, and in processing conditions for the preparation of stable HT-ST sterile milk concentrates.

Possible relationship of genetic variants of milk proteins to the processing properties of milk is recognized. However, in the case of two  $\alpha_s$ -caseins, designated B/B and C/C, no significant difference in the heat stability and gelation resistance was observed in sterile concentrates containing these variants. Previous work had established the interrelationships of the caseins, phosphate and calcium to gelation and sedimentation in concentrated milks. Further basic research has added important information on the mechanism of this process. The procurement and installation of improved pilot plant equipment during the next report period will extend the study of production variables influencing the physical stability of sterile fluid concentrates containing select chemical additives. The significant factor has been found to be the level of phosphate in the system. The gelation process appears to consist of an interaction between orthophosphate and some internal group of the caseinate particle. As a consequence of this interaction the particles unfold and the unfolded residues are bridged together by calcium into a three dimensional network. The group or groups within the caseinate particle with which phosphate interacts have not been identified. An accurate description of the gelation process should establish guidelines for a rational processing of concentrated milk products so as to avoid gelation.

Under one of the P.L. 480 research grants at the National Institute for Research in Dairying, Reading, England, cow's lipases were emphasized in work done in 1962. Purification of the original extract to a point where

the lipolytic activity had increased 15 times was achieved. Since lipolytic problems in milk may result from high concentration of lipases in only a few animals, many individual milks will be tested.

Under the P.L. 480 research grant at the Freddo Experiment Station, Milan, Italy, new food products, to be prepared by combining concentrated milk and fruit juices, are being investigated. The expected coagulation of casein occurs except with quinces which are high in pectin. Search for preventatives of coagulation has shown that pectin and carboxy methyl cellulose are effective inhibitors while alginates and gelatin are ineffective.

Under one of the P.L. 480 research grants at the National Institute of Agronomic Research, Paris, France, non-protein nitrogen substances which are formed from milk proteins during various industrial treatments of milk are being investigated. Several electrophoretically distinct components were observed in the supernatant after coagulation of kappa-casein with rennin; the rennin-sensitive linkage of kappa-casein is probably an ester between phenylalanine and a hydroxyl group, probably part of an amino alcohol or glucide.

#### B. Dry whole milk and other dried milk products.

Development of both the vacuum and spray processes for drying whole milk in the form of foams has continued. Several plants have installed the equipment for foam spray drying condensed skim milk and other milk concentrates; one large new plant is designed especially for the foam spray process.

1. Continuous vacuum foam drying. A reproducible set of operating conditions has been developed for "winter milk" which produces a high quality dry whole milk at a rate about 20% higher than the estimated requirement for economical commercial operation. With suitable material thus available, storage tests were begun in which the dry milks are made, handled and packaged with little or no contact with oxygen. For these tests storage is at ordinary refrigerator temperature (40°F.) and at room temperature (73°F.). Residual oxygen content in the cans is less than 0.05% by volume, and oxygen scavengers have not been employed.

Concurrently, statistically designed experiments directed toward optimization of the process are being conducted in the integrated pilot plant. The process must be optimized with respect to 14 currently recognized variables. Thirteen of these variables are controllable. They may be divided into two groups, viz., those characterizing the dryer feed and those representing the drying conditions. Study of the 13 controllable variables for process optimization is simplified by isolating them from the fourteenth (seasonal) variable. However, they can then be studied only during periods of constant foam stability (winter and summer). The seasonal effects must be investigated by comparative studies among the seasons. Since any one seasonal period is relatively short, this investigation must be restricted to that group of variables most closely related to feed characteristics. Moreover,



all pilot plant studies are necessarily coordinated with the laboratory foam stability and composition tests.

From present information there are four distinct seasons with respect to foam behavior in the continuous vacuum dehydrator, i.e., summer, winter and the two transitional periods. The winter season lasts approximately 13 weeks; the spring transition, 8 weeks; summer, 23 weeks; and fall transition 8 weeks. During the winter (low foam stability) season and the summer (high stability) season the foams remain constant in behavior, whereas during the transition periods they change almost daily.

Although a definite statement cannot yet be made, it appears likely that the production rate required for economic feasibility may be attainable even with the more difficult "summer milk" supply. This advance has come about largely through the use of phospholipid (soybean lecithin) as a minor additive to the milk. The most important potential problem associated with phospholipid additive to control foam behavior despite seasonal changes in milk characteristics appears to be that different additive levels may be required at different seasons - unless a level of phospholipid can be found which will mask all seasonal differences. Laboratory research has been expanded to determine whether such a level exists or, if not, what levels of added phospholipid are required throughout the year. This research will be coordinated with studies in the continuous drying plant.

Laboratory observations of milk concentrate foams during the past fall showed the anticipated decrease in foam stability. During this period a rise was noted in the phospholipid content of the milk. Whether there is a cause and effect relationship between foam stability and natural phospholipid level is not certain from available information.

2. Spray foam drying. With nitrogen, air or carbon dioxide injection fresh spray dried whole milk has excellent flavor. The product from nitrogen or air tends to float on water and stirring is required for rapid dispersion; carbon dioxide injection yields a product which reconstitutes more readily and resembles the vacuum shelf dried powder. The nitrogen- and air-prepared samples on storage with 5 percent hydrogen in nitrogen, plus a catalyst in a separate porous container, maintain good flavor for 6 months or more; the powder prepared with carbon dioxide deteriorates more rapidly, presumably because the carbon dioxide is irreversibly absorbed by the powder, resulting in a vacuum within the container thus causing air to leak in during storage.

Foam drying continues to look promising for preparing easily dispersible whole milk powder of beverage quality. Some technical problems remain, but there is confidence that they will be solved. Further improvement in flavor stability during storage is desirable.

3. Flavor. Further investigation of milks made from nonfat solids and butter oil tends to substantiate the hypothesis that chemical changes in the fat phase are largely responsible for stale flavor development in whole milk powders. (This generalization may be limited to powders that have not been

damaged by heat during preparation, that have low residual water content, and are maintained in an oxygen-free atmosphere.) By molecular distillation the off-flavors developed during long storage were removed from nitrogen-packed butter oil. When combined with skim milk the treated butter oil gave a good flavored whole milk. This molecular distillation treatment should be valuable by providing a concentrate of stale flavor components for chemical identification and analysis.

An exhaustive study of ketone formation was conducted. Methyl ketones have been regarded as likely sources of storage off-flavor in whole milk powder. It was found that water is essential for ketones to form in milk fat under the influence of heat. Chemically dried milk fat was very stable to heat in respect to ketone formation. The precursors of methyl ketones have been isolated and identified tentatively as triglycerides that contain a series of beta-keto fatty acids. Water appears to have a catalytic role; it is involved in the hydrolysis of beta-keto acids from the glycerides, a step which is prerequisite to decarboxylation and methyl ketone formation.

A 6-month old foam spray dried whole milk which had been nitrogen-packed and stored at 105° F. was analyzed for carbonyls. No free aldehydes, saturated or unsaturated, could be detected. No dicarbonyls and only a trivial amount of methyl ketones were found. Thus it is possible to virtually eliminate carbonyls as the source of off-flavor in milk powder stored in the absence of oxygen. The relationship to stale flavor of lactones, produced spontaneously from precursors normally present in milk fat, is now being determined.

An oxygen level of essentially zero, which inhibits oxidized flavor development was maintained for 6 months in cans by means of a hydrogen-palladium oxygen scavenging system. This was not achieved with flexible film packages.

Two sets of 50 whole milk powder samples were judged for flavor by a student preference panel at Oregon State University and were scored for type and magnitude of flavor defects by trained panels at Oregon State University and in the Washington and Wyndmoor laboratories engaged in the milk drying research. It has been concluded that the average total score from each of the trained panels will predict satisfactorily the consumer preference; the trained panels' scores for individual flavor defects did not correlate satisfactorily with preference scores.

4. Structure and dispersibility. The film that spreads spontaneously on water from milk powders made with the entire milk fat is being collected in quantities sufficient for a detailed study of its composition. Work on this film material is being intensified since the discovery that powders made from milk containing selected fat fractions do not form surface films which hinder the wetting of the powder mass.

The effect of powder permeability to gases on the flavor stability of spray dried whole milk foams is being investigated. It has been noted that powders produced and packaged under seemingly identical conditions may vary markedly



in flavor stability. The permeability of these powders has also been found to vary. A correlation between these factors is being sought.

Studies of spray dried whole milk foams using gas adsorption techniques indicate that the structure is a gas-in-solid foam characterized by a very low order of surface porosity. This type of structure improves the dispersibility of milk solids but prevents the material from sinking rapidly through an air-water interface. Ideas for producing spray dried particles of the desired type will be tested.

Foam spray dried powders were made from milks containing the individual recently discovered genetic variants of calcium-sensitive casein. No initial differences were noted in the gross characteristics of the milk powders.

5. Other dried milk products. A considerable commercial interest has developed in foam spray drying as a one-step means of making an instant nonfat dried milk. An important aspect of the process is the greatly increased plant capacities made possible because concentrates up 60% solids can be dried. The usual maximum is 45% solids.

The foam spray drying method of making readily dispersible powdered milk has been applied successfully to the preparation of additional dried products such as ice cream mixes, malted milk and cocoa-flavored sweetened skim milk.

It has been discovered that the use of a mixture of compressed nitrogen or air and carbon dioxide results in the production of lower bulk density than when nitrogen or air alone is employed. This means that concentrates which cannot be dried using air or nitrogen injection may be dryable if a mixture of either of these gases and carbon dioxide is used.

#### C. Improved butter and milk fat products.

Milk fat is a serious surplus problem for the dairy industry; its high price in comparison with competing fats for household spreads is a major reason behind this situation. Research to improve the desirable qualities of butterfat is essential. The Dairy Research and Marketing Advisory Committee has recommended that "basic scientific studies on butterfat should be expanded with the view in mind of defining the various fractions and studying the properties and combinations thereof. It is further suggested that this study include the unique flavor characteristics of butterfat and the potential uses of the various components. Such studies should also include the possible intensification of butter flavor in foods containing butterfat or its fractions."

Development of oxidized and stale flavor in milk fat is a serious storage problem. To date no successful edible antioxidant has been discovered which will prevent oxidized flavor in butter, although antioxidants are widely used in competing household spreads. In concentrated sweetened cream deaeration plus ascorbic acid significantly extended the shelf life of this product at

room temperature. Possible application of this discovery to butter needs further investigation.

Stale flavor in anhydrous milk fat can be removed by steam stripping at temperatures near 200°C.; evidence indicates that precursors of stale flavor are also removed by this treatment, thus suggesting that development of stale flavor during storage can be prevented.

#### D. Improved ripened cheeses.

That Cheddar cheese made from raw milk has a more intense flavor than cheese made from pasteurized milk is well known. In contract research at Ohio State University chemical differences in the two types of cheese are being investigated. Appreciably greater amounts of free 5-carbon and higher fatty acids have been found in Cheddar cheese made from raw milk. Cheese flavor was not affected by the addition of hypoxanthine, uric acid, acetaldehyde, or cysteine to either raw or pasteurized milk. Improvements were made in analytical techniques for the volatile components of cheese. Through the use of radioactive sulfur it was established that larger amounts of volatile sulfur compounds are present in raw milk cheese.

A low fat cheese of good flavor and body should find a ready market with today's diet conscious consumers. A skim milk cheese with typical Cheddar flavor could not be prepared. Skim milk with enough added fat to give 3 to 4 percent fat in the cheese has been successfully made in the pilot plant into a product with satisfactory body, texture and mild flavor. Some fat is necessary for the Cheddar type flavor; an additive such as a monoglyceride is necessary for the desired body and texture. Additives such as pectin, gum or gelatin improved body, but contributed an undesirable flavor. Proteolytic enzymes, either bacterial or animal in origin, cause bitterness in the aged cheese. Making a good low fat cheese posed new problems both in technology and in microbiology; that is, the standard cheese cultures and making procedures both had to be modified. There is good reason to believe that a satisfactory low fat cheese with Cheddar type properties can be manufactured.

Under a P.L. 480 research grant at the National Dairy Research Institute, Karnal, Punjab, India initial experiments with over 100 isolates of molds, yeast and bacteria to test their potential production of milk coagulating enzymes for use in cheese manufacture, showed that a few molds give positive results when grown on wheat bran extract medium and a few of the spore forming bacteria when grown in milk based media.

Research under a P.L. 480 grant with the Institute of Biochemistry, Turku, Finland showed that the inhibiting effect of serine on growth of Streptococcus thermophilus is believed to be due to its stoppage of isoleucine synthesis. Experiments with other microorganisms indicate that serine decreases their production of the enzyme threonine dehydrase.

Under a P.L. 480 research grant at the National Institute of Agronomic Research, Paris, France, a clotting process on treatment of milk with rennin



is being investigated. The reaction which takes place with individual components of casein will be included so that the information can be applied to improving cheese.

The Institute of Dairy Industry, Warsaw, Poland under a P.L. 480 grant has investigated the possible increased synthesis of vitamin B through the use of mutant strains of molds in cheese manufacture. X-rays and gamma rays were used to produce mutations of P. candidum and roqueforti. The promising mutants are being investigated to determine their potential usefulness in cheese manufacture.

The National Institute for Research in Dairying, Reading, England has been studying the application of partition chromatography and allied techniques to the differentiation of microorganisms important in dairy products, under a P.L. 480 grant. By the use of punched cards some correlation was observed between the free amino acids occurring in the cells of cultured bacteria and the quality of the cheese from which the bacteria had been isolated. Some correlation between species and the amino acids present was also observed. Bacteria which contain relatively large quantities of threonine and observable amounts of glutamine were more common in better cheese; also relatively low amounts of aspartic acid and lysine are found more often in these bacteria than in those from the poorer cheese. In relation to the general problem of bacterial flora and cheese quality it is unlikely that a simple survey will do more than suggest a few possible lines of further inquiry.

#### E. Flavor components and flavor stability.

1. Chemistry. For years researchers in milk flavor problems have felt that carbonyl compounds contribute an important part in undesirable flavors in dairy products. Their formation was explained on the hypothesis that unsaturated fats are oxidized to unstable hydroperoxides which in turn decompose into aldehydes and ketones. Considerable chemical evidence supports this mechanism. Identification in Australia of vinyl n-amyl ketone as a major compound responsible for oxidized flavor in milk fat was an important event in the chemistry of undesirable milk flavors.

The formation of methyl ketones is believed to result from a different mechanism;  $\beta$ -keto glycerides in the presence of moisture can slowly hydrolyze to the free  $\beta$ -keto acids which spontaneously decompose into methyl ketones, more rapidly at higher temperatures. Especially dried milk fat does not produce these compounds even on heating. Qualitatively, saturated ketones do produce some flavor effects in dairy products when present in sufficient amounts; quantitatively, more detailed experiments must be run to decide if these compounds have any influence in dairy products under normal conditions. Lactones in milk were identified several years ago and described as producing a coconut type flavor. Here too, in a quantitative sense, more refined experiments with pure compounds are needed. Much information is available to show that in the complete absence of oxygen stale flavor develops in milk powder. Research in several laboratories indicates that the same or similar compounds are responsible for this stale flavor in both dried whole milk and

evaporated milk; although NFDM also develops a stale flavor on long storage, it is believed that the major source of this flavor is milk fat; skim milk may well contain enough fat to account for this stale flavor. Steam stripping or high vacuum treatment near 200°C. will remove the stale flavor from milk fat; the precursors of this flavor may also be removed.

2. Taste panel. From a contract with Oregon State University, including cooperation of three expert taste panels (Oregon State University, Dairy Products Laboratory in Washington and EU in Wyndmoor) it can be concluded that consumer acceptance of reconstituted dry whole milk can be predicted by well trained "expert" panels. Analysis of the data showed that the average score can be used as a basis for a scoring system which will predict consumer acceptance. However, lack of agreement on description of individual flavor difference of a single sample by different "expert" panels, or even by individual members of the same panel, shows that some problems remain to be solved.

Under a P.L. 480 research grant with the Biochemical Institute, Helsinki, Finland, Professor Virtanen has published about 50 papers based on work done under this agreement. These include important contributions to milk chemistry. Considerable progress has been made toward synthetic diets for cows; if this is achieved many of the problems of milk properties during processing can be attacked more scientifically. Studies of sulfur and selenium metabolism in plants of the onion family are progressing nicely through use of radioactive sulfur and selenium. Enzymatic actions on thioglucosides of the Cruciferae family are being investigated; some of these reactions proceed rapidly in the neighborhood of 0°C. The potential goitrogenic effect of milk on humans if the cow's diet contained large amounts of Brassica plants has been disproved conclusively with experiments on rats.

#### F. Isolation, structure and properties of milk proteins and the interactions of milk components.

Three genetic variants of  $\alpha_s$ -casein were isolated and partially characterized. Two ribonuclease species in milk were identified by chromatography; one was obtained in pure form and shown to be identical with commercial bovine pancreatic ribonuclease.

A new crystalline protein, named for convenience "lactollin," has been isolated from milk in very small amounts by chromatography on DEAE cellulose. It is a basic protein with a minimum solubility and isoelectric point of about pH 8 and a molecular weight of 43,000. Its amino acid composition is unusual in that it contains no methionine and very little alanine and a large amount of aromatic amino acids.

The red protein of milk, isolated several years ago, has been further characterized by determining its amino acid composition. The distribution of amino acids in this protein shows few unusual features. The basic nature of the protein is, however, reflected in the finding of 25 more cationic than



anionic groups. The red protein was shown to be different from bovine serum transferrins in molecular properties and amino acid composition.

Continued progress is being made in elucidating the amino acid sequence of  $\alpha$ -lactalbumin. The amino acid sequence of four pure peptides comprising about one-fourth of the molecule has been completed. With the experience gained in the purification and analysis of these peptides, as well as the improved equipment now available, it is expected that the amino acid sequence of  $\beta$ -lactalbumin will be completed in the not too distant future.

Recent investigations have shown that  $\beta$ -casein occurs in several genetically controlled molecular forms.  $\beta$ -Casein was prepared from the milk of individual cows as well as from pooled milk. Amino acid determinations of these individual  $\beta$ -caseins have not clearly revealed a unique amino acid difference for these forms of  $\beta$ -casein. Another approach to the problem of the genetic forms of  $\beta$ -casein has been investigated. Thus, the large peptides produced by the action of trypsin on  $\beta$ -casein prepared from pooled milk were purified. One of these peptides was found to be present in two homologous forms differing by one histidine residue. The milk from individual cows was found to contain either one or both of these forms. The conclusion from these experiments is that the histidine content of  $\beta$ -casein varies in the genetic forms. Further work is needed, however, to completely clarify the differences in the amino acid composition in the genetically different forms of  $\beta$ -casein.

The antigenic mucoprotein which was isolated from the fat plasma interfacial region of milk has been studied further. Proteolytic digestion of this material, followed by partial purification on Sephadex and ion exchange columns, has given a glycopeptide fraction with a combined hexose, hexosamine and sialic acid content of over 50 percent. The crude digests, resulting from the treatment of interface material with trypsin, chymotrypsin and papain, have strong activity in precipitating antibodies prepared in rabbits against the original mucoprotein. The diffusion technique of Ouchterlony has demonstrated that the digests, like the original mucoprotein, are not immunologically pure. The preparation of a partially purified degradation product of the milk fat/plasma membrane mucoprotein, with immuno- and physico-chemical properties of the interfacial material itself, greatly simplifies a study of changes in these properties during milk processing.

#### G. Removal of radionuclides from milk.

Research on the removal of fallout from food, especially milk, continues to be a popular subject with public and political overtones. Further important progress has resulted from the joint effort of the Atomic Energy Commission, Public Health Service, and U. S. Department of Agriculture, particularly toward a demonstration of commercial scale use of the process for strontium 90 removal. A contract for this purpose has been negotiated.

The nutritional quality of milk, processed for removal of strontium with the fixed-bed ion exchange method developed at Beltsville, was evaluated by determining growth rates on baby pigs and rats, and by chemical analyses.

The results showed no significant differences between the animals fed ion exchange processed milk and those fed normal milk; there were no marked changes in the vitamin contents of the two milks.

Studies on resin regeneration show that about three fourths of the regenerant (mixed salt solution) can be profitably reused; thus the process requires only about five resin bed volumes of fresh regenerant per 35 volumes of milk. Total cation resin processing costs have been estimated at 0.48 cents and 0.92 cents per quart of milk when technical grade or U.S.P. grade salts are used for resin regeneration, respectively. Investigations were made to avoid acidification and neutralization, which results particularly in increase of the potassium content of the treated milk. Laboratory tests have shown that acidified milk may be successfully neutralized with a combination of calcium hydroxide and sodium hydroxide. A two-step resin treatment procedure which requires no additives for the adjustment of pH was discovered. Milk passed through a sodium charged resin in the "continuous" contactor resulted in about 95 percent removal of strontium. Readjustment in calcium, magnesium, and potassium concentration in the milk was made by passing the treated milk through resin charged with these cations. Flavor of the treated product was acceptable.

In related developments the Civil Defense Division, Department of Defense, is investigating through a contract the feasibility of ion exchange membrane electrodialysis for removing radionuclides from milk. Representatives of industry and government met in October, 1962, to consider the potential usefulness of various methods of removing both cationic and anionic radionuclides from milk. The procedures discussed included ion exchange (column and moving bed systems), membrane electrodialysis and calcium phosphate precipitation.

The principal effort on pilot plant investigations at Beltsville will be to test the recently installed automated equipment which was designed as a complete unit. Conditions for automatically adding acids and neutralizers, and for controlling the sequence of cycles, will be determined. Further evaluation of the milk quality, flavor, removal efficiency, and regeneration requirements will be made. Information from laboratory studies will be used to develop a procedure for the removal of iodine 131 as an integrated part of the decontamination process.

One of the important questions from the public and dairy industry is how soon can milk plants install the equipment. A vital factor in this decision is beyond the responsibility of Utilization Research; government agencies responsible for the safety of food must first decide when treatment is necessary.

#### H. Control of spoilage.

Bacterial spores, which are responsible for the high temperature treatment necessary to avoid spoilage in canned foods, yield their secrets slowly. Four species of bacterial spores were subjected to extensive electrodialysis.



Gas adsorption and displacement methods were used to determine the surface areas and true densities of three varieties of spores. The data suggest that dipicolinic acid is not held in the intact spore by ionic bonds accessible to water. Studies support the hypothesis that the spore structure can be penetrated by both water and large dissolved molecules. There seems to be little or no apparent correlation between physical surface features and heat resistance. However, large pores seem to exist in the spore wall. Visible verification of spore surface openings and, if possible, their size and number, is being sought with electron microscopy.

This research program on bacterial spores is furnishing basic information on the surface porosity, permeability and adsorbability of spores; and on their content of dipicolinic acid and how it is released on germination. A better understanding of sporulation and germination may lead to a practical means for sterilizing milk and other foods at a much lower temperature than is now possible.

### I. Genetics and milk composition.

Recent reported progress in the chemistry and heredity of the lower forms of life suggests that this area may also be fruitful in the search for better milk products. Techniques to produce sometimes useful mutations in micro-organisms are already known. To gain control of milk properties through genetics will take a longer time since the generation cycle is in years rather than days or weeks as it is in bacteria.

Investigation of the genetic complexity of the caseins has continued under an examination of caseins from 1,000 cows, including Holstein, Jersey, Guernsey, Brown Swiss, Ayrshire, and Shorthorn breeds. Three types each of  $\alpha_s$ - and  $\beta$ -casein are now recognized. These variations are believed to be genetically controlled, as was previously established for the  $\beta$ -lactoglobulins.

Many of the problems in milk processing have never been solved satisfactorily on a year-round basis; processing procedures developed by the trial and error approach have given the dairy industry practical results, but sometimes there is more art than science in the milk plant. Occasionally suspicion has arisen that one or more individual cows may excrete some factor in their milk which has a deleterious effect on processing characteristics, such as heat stability.

Under a P.L. 480 research grant with the Research Center on Macromolecules, Strasbourg, France on kinetics of initial degradation of deoxyribonucleic acid (DNA) by acid deoxyribonuclease it was shown that the reaction occurs according to "single strand" kinetics; this is the first known example of this type of action. Acid deoxyribonuclease from hog spleen was obtained in pure form. Chromatographic studies of DNA have resulted in separation of the denatured substance from the native material.

## J. Allergens of milk and other agricultural products.

One human source of strongly reactive antiserum (Acton) and one moderately reactive serum have contributed to development of the program on milk allergens this past year. The high reactivity of the Acton serum with the milk protein  $\alpha$ -lactalbumin indicates that either the protein itself is the allergen or the presence of an allergenic impurity in the protein. Collaborative studies with the Department of Immunochemistry at the Walter Reed Army Medical Center have continued on the immunological characterization of the mucoprotein from the fat-plasma interface of cow's milk.

In a collaborative program with Jefferson Medical College investigations on the fundamental basis of milk allergy in infants are being made to ascertain the conditions which lead to the formation of antibodies to cow's milk. Some of the initial problems have been resolved. For example, only a very limited amount of blood can be safely withdrawn from newborn infants. Serum or plasma must be used to assay the milk allergens; plasma is preferable because a greater volume of it can be recovered from blood. Therefore, studies were conducted on the suitability of using plasma in place of serum for the hemagglutination and passive transfer tests which are essential in the tests for milk allergens; it was concluded that heparinized plasma also may be used.

Fractionation and immunochemical characterization of the castor bean allergens have continued. Gel filtration is being used for fractionating the allergenic material; the Ouchterlony double diffusion test is used to determine precipitinogenic capacity of each fraction. Studies of the enzymatic and chemical degradation of the allergen show that it is hydrolyzed to the same extent by the enzymes pepsin and trypsin, but the loss of precipitinogenic activity is greater with trypsin. Hydrolysis with pronase is much more extensive than with pepsin and trypsin, and the loss of precipitinogenic capacity is almost complete. Sodium borohydride, which reductively cleaves proteins at their cystine linkages, completely inactivates the allergen as judged by precipitinogenic capacity.



PUBLICATIONS AND PATENTS REPORTING RESULTS  
OF USDA AND COOPERATIVE RESEARCH

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## III. MARKETING AND ECONOMIC RESEARCH

DAIRY PRODUCTS - MARKET QUALITY  
Market Quality Research Division, AMSProblem.

Modern marketing practices in the dairy industry have intensified the problems of detecting inferior lots of milk and of increasing the storage life of dairy products. Several kinds of stored-product insects and mites damage or contaminate dairy products and cause extensive losses, principally in nonfat dry milk and cheese. The losses are important not only to the dairy industry but also to the Department in connection with its price support and storage program. To maintain quality of these products in marketing channels, research is urgently needed on the factors influencing keeping quality, and on the development of safe and effective procedures for controlling and/or preventing infestation in warehouse storage, and during transportation by rail, as well as by packaging to provide better protection for nonfat dry milk against infestation. New and improved objective quality tests are also needed for bulk milk and other products. A related problem is the occasional presence of antibiotic and pesticide residues for which improved detection methods are needed.

## USDA PROGRAM

There is a continuing program of basic and applied research aimed at developing new and improved methods for assessing the important quality factors in a variety of dairy products. Work is being done at Beltsville, Maryland, on the development of simple, rapid screening tests for detection of chlorinated insecticide residues in dairy products and on the stability of anhydrous butterfat in storage. A 2-year contract study with the University of California at Davis on the estimation of protein content of milk by dye-binding is nearing completion. A 3-year contract with the University of Minnesota was initiated during the past year to study the quality of milk used for manufacturing purposes. The Federal scientific effort devoted to research in this area, objective measurement and evaluation of quality, totals approximately 2.0 professional man-years.



Study of the types of bacteria responsible for the deterioration of market milk (E8-AMS-2(a)) was terminated during the period.

There is a long-term program involving entomologists and chemists engaged in basic and applied research on the prevention of insect infestation and contamination of dairy products in the marketing channels. Cooperative work with the Wisconsin Agricultural Experiment Station, the Commodity Credit Corporation, and industry groups is included. In addition to work specifically aimed at insects attacking dairy products, previously done at Madison, Wisconsin, studies applicable to these and other insects as well are conducted at Savannah, Georgia, and are reported in Area 13, "Insect Control in Marketing Channels."

The Federal scientific effort in the insect studies totals 2.0 professional man-years divided as follows: basic biology and ecology 0.6; insecticide evaluation 0.5; insecticide residues 0.1; insect-resistant packaging 0.6; and program leadership 0.2.

Toward the end of the reporting period the work at Madison was relocated at the stored-product insects station at Fresno, California, as part of a Branch action to consolidate several small field stations. This will permit more effective utilization of manpower and equipment.

Line project MQ 1-4, dealing with the control of dermestid beetles in dry-milk processing plants, was terminated with completion of the major objectives of the project.

## REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

### A. Objective measurement and evaluation of quality

1. Pesticide Residues. Work was completed on the development of two chromatographic techniques for separation of pesticide residues from fats and other interfering materials (cleanup) using, respectively, carbon-celite, and Florisil. By use of these chromatographic techniques, the sample can be prepared for analysis by paper chromatography in one step where formerly two or more steps were required. Nearly all common chlorinated insecticides can be separated from interfering materials by these procedures so that a reasonably complete screening of samples for pesticide residues can be made in one operation. The Florisil cleanup is especially

useful for samples containing butterfat. Carbon-celite cleanup can be used in a few cases for insecticides in butterfat which cannot be recovered from Florisil, but it is more satisfactory with other agricultural products. The use of the chromatographic procedures for cleanup of samples for colorimetric determination of DDT was also studied but a number of problems in the overall procedure were encountered and these are still under study.

(MQ 3-11)

2. Protein Content. This study on the estimation of the protein content of milk by dye-binding is being carried out under contract with the University of California at Davis. Amido black was selected as the most satisfactory dye for this purpose. It is available commercially in a form relatively free from interfering materials, it gives a greater change in optical density per unit of protein and the dye-protein complex can be removed by centrifugation. The dye-protein reaction was studied in detail. The relation of protein to dye bound by protein was non-linear. This may also be the case with samples of mixed herd milk collected in the field. Mastitic milk differed significantly from normal milk in dye-binding capacity.

(MQ 3-14(c))

3. A Study of Methods for Grading Milk. A survey of the quality of milk for manufacturing was initiated under contract with the University of Minnesota. Samples of both can and bulk handling are being collected during all four seasons from three major producing areas, Iowa-Minnesota-Wisconsin; Missouri-Kentucky-Tennessee; and the Boise Valley in Idaho. Bacteriological quality will be evaluated from plate counts and reduction tests which will be related as far as possible to farm practices in order to provide a basis for setting up grading standards. Spring and summer sampling has been completed for Iowa-Minnesota-Wisconsin and summer sampling for Missouri-Kentucky-Tennessee. No analysis has been made of data obtained thus far.

(MQ 3-44)

4. Bacteria in Market Milk. The final report on this project was received during the reporting period. The study was concerned with the types of psychrotrophic bacteria found in market milk. A "key" method was developed using a computer to sort bacterial isolates into nine groups on the basis of a limited number of tests. Pseudomonads were found to be of major importance in spoilage of milk at refrigerator temperatures. Alcaligenes species and



enterobacteria were also involved. Rapid spoilage was associated with the simultaneous development of several strains of bacteria and slow spoilage occurred when one or rarely two strains of bacteria gained dominance. In the former case, flavor defects were poorly defined, but under the latter evaluations, one or two specific flavor defects developed after relatively long storage time.

The results obtained in this study provide a theoretical basis for predicting keeping quality of market milk. However, no practicable method of applying this information to routine testing is yet available.

(E8-AMS-2(a))

## B. Prevention of insect infestation

1. Basic Biology and Ecology. A significant discovery is that insects infected with a pathogen fluoresce a bright yellow-green when irradiated with ultraviolet light in the 366 m $\mu$  band. The phenomenon has been demonstrated with Trogoderma glabrum, T. inclusum, and the Indian-meal moth. The infective organism has been identified as the Schizogregarine protozoan, Mattesia dispora Naville. Using a Beckman DK-2 recording spectrophotometer, it was determined that the emission spectrum of an aqueous suspension of the spore stage of the protozoan was 512.3 m $\mu$  with a 363 m $\mu$  source lamp. This discovery provides a useful tool in the study of the disease and in its control in laboratory cultures. It should stimulate interest in biological control studies with this highly pathogenic organism. A technique was developed for obtaining Trogoderma larvae free of the disease. Eggs obtained by using a special oviposition chamber are sterilized with mercuric chloride and alcohol, then transferred to sterile jars and rearing medium.

(MQ 1-4)

Further studies have been made with the sex attractant produced by unmated adult females of the black carpet beetle and two species of Trogoderma, glabrum, and inclusum. Adult males have been found to respond to this attractant in a characteristic pattern. The characteristic features of response are a forward and upward extension of the antennae, a zigzag pattern of movement, and elevation of the fore part of the body. Evidence was obtained that the receptors are in the male antennae. The attractant has been collected on absorbent paper discs over which unmated females have walked, by an ether wash of filter paper and glass jars that have held the

females, or by filtering it from an air stream passed through a flask containing the females. A simplified method has been developed for collecting the attractant on absorbent paper discs, and for handling and bio-assaying the discs.

(MQ 1-32)

2. Insecticide Evaluation. Laboratory studies showed that all black carpet beetle larvae were killed after a 7-day continuous exposure on a malathion residue that had aged 4 weeks after application over a special floor finish or a traffic paint that had been put on concrete. Other treatments tested were ineffective in preventing the almost immediate breakdown of malathion on concrete surfaces. This is a serious problem in warehouses and milk processing plants.

(MQ 1-4)

Dermestid beetle larvae were exposed for 4 or 6 hours continuously, or for 2 hours at 24-hour intervals for a total exposure of 4 or 6 hours. This was to determine the effect on mortality of intermittent versus continuous exposure to malathion residues of 100 mg. per sq. ft. The average mortality was higher for the larvae exposed continuously.

(MQ 1-4)

The techniques and equipment for conducting laboratory mite fumigation tests were modified, improved, and evaluated. Unfortunately there are no established, proven procedures to follow in this work. The new method uses 12-liter boiling flasks for test chambers and magnetic stirrers to equalize the distribution of fumigant. A new mite handling technique uses a low temperature counting plate to eliminate the harmful effects of CO<sub>2</sub> as an anesthetizing agent.

(MQ 1-6)

A proportionate share of the research on insecticide evaluation at Savannah, Georgia, has been charged to the work on dairy products. Although many of the results are directly applicable, it is not feasible to report only certain portions here, or to include all the information under each of the several appropriate commodity areas. Therefore, the entire report is included in the cross-commodity report in Area 13.

3. Insecticide Residue Analysis. As with insecticide evaluation, the cross-commodity residue analysis work at Savannah, Georgia, is reported in Area 13.



4. Insect-Resistant Packaging. An additional packaging test has been completed in which different closures for multiwall paper bags containing nonfat dry milk were compared for their ability to prevent insects from invading the bags. Examinations were made after 1, 3, 6, and 12 months of storage during constant exposure to dermestid beetle infestation. An experimental hand-applied tape-over-stitching closure gave the best results. Stitching-over-tape and the normal sewn-open-mouth closures were less effective in the order named.

(MQ 1-13)

Additional cross-commodity packaging work conducted at Savannah, Georgia, is reported in Area 13.

Based on the results of several years of research on insect-resistant packaging, recommendations were made to CCC for improved closures on the packaging for nonfat dry milk. Specifications were worked up with the assistance of an industry association technical committee and have been adopted by the Department. Purchases are now being made under these specifications and it is anticipated the serious problems of insect infestation in storage encountered during the past several years will be considerably alleviated.

(MQ 1-13)

## PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

### Objective Measurement and Evaluation of Quality

Moats, W. A. 1963. One-step chromatographic cleanup of chlorinated hydrocarbon pesticide residues in butterfat. II. Chromatography on Florisil. Journal Association Official Agricultural Chemists, 46: 172. (MQ 3-11)

### Prevention of Insect Infestation

Hilsenhoff, William L., and Dicke, Robert J. May 1963. Effects of temperature and humidity on cheese mites--with review of literature. USDA Marketing Research Report No. 599, 46 pp. (MQ 1)

Marzke, F. O. 1963. Food preference studies with Trogoderma inclusum, a pest of the dry-milk industry. Journal of Economic Entomology, 56(1): 109. (MQ 1-4)

DAIRY PRODUCTS - MARKETING FACILITIES,  
EQUIPMENT AND METHODS  
Transportation and Facilities Research Division, AMS

Problem. A survey of market milk and ice cream plants throughout the country, by the University of Illinois in 1958, shows that the equipment, work methods, and facilities of many of these plants are obsolete and the production per man-hour employed relatively low. A major factor contributing to this obsolescence is the development during the last few years of new types of equipment which can be brought under automatic control. Because of the investment required and the uncertainties of the returns they would obtain, plant operators have been reluctant to shift to automated equipment on a piecemeal basis. They also have been reluctant to build new plants because of a lack of guidelines and criteria on automated plants. However, studies indicate that it is possible in fully-automated plants to increase the productivity of labor 100 percent or more, to improve the qualities of the finished products, and to develop better management-employee relations. Engineering layouts and operating criteria therefore are needed for automated plants to provide guides to plant operators in making the shift from their present equipment and facilities. Most dairy plants lack the technological and engineering skills necessary to plan and develop suitable plant layouts and designs, or to select the types of equipment needed and the controls necessary for full automation. Automated equipment and processes for some types of dairy plants still largely are lacking or are nonexistent. Therefore, engineering research also is needed to develop equipment and processes for automating these plants in order to increase labor productivity and improve product quality.

USDA PROGRAM

The Department has a continuing long-term research program involving industrial engineers, mechanical engineers, and dairy technologists engaged in applied research to develop improved methods, equipment, operating criteria, and plant layouts for dairy plant operators. Also, some work is being done

Current research, conducted under contract by Paul H. Tracy, DeLand, Fla., covers the development of layouts and operating criteria, based on current technology, for automated dairy product plants. It features the use of remotely operated valves, electronic-controlled devices, and highly mechanized equipment. Work in the Washington office consists of checking, organizing, and preparing for publication the series of reports prepared by the contractor.

The Federal effort devoted to research in this area is 1.4 man-years; 1.0 man-year for direct work and 0.4 man-year for program leadership.



## REPORT OF PROGRESS OF USDA AND COOPERATIVE PROGRAMS

A. A Procedure for Planning the Most Efficient Use of Dairy Manufacturing Plant Resources, Including Labor and Facilities

At Lafayette, Ind., in cooperation with the Purdue University Agricultural Experiment Station, a manuscript entitled "Simulation Procedures for Production Control in an Indiana Cheese Plant" was completed and published.

B. Layouts and Operating Criteria for Automated Dairy Product Plants

At DeLand, Fla., work under a research contract covering the development of layouts and operating criteria for six different types of automated dairy product plants was completed. The six types of plants and the status of work on the publication of these reports are as follows:

1. Special Purpose Milk Plants (Market Milk and Half-and-Half). A manuscript entitled "Layouts and Operating Criteria for Automation of Dairy Plants Processing Milk and Half-and-Half" was completed and published.
2. Multipurpose Milk Plants (Market Milk, Half-and-Half, Coffee Cream, Buttermilk and Chocolate Drink.) A manuscript entitled "Layouts and Operating Criteria for Automation of Dairy Plants Processing Milk, Half-and-Half, Coffee Cream, Buttermilk, and Chocolate Drink" was completed and published.
3. Ice Cream Plants. At the end of the year, the contractor's report covering layouts and operating criteria for automated and highly mechanized ice cream plants is in the process of being reviewed and prepared for Department publication. The report shows that an ice cream plant handling 200,000 gallons annually, using automated equipment and an improved layout, could reduce its annual labor costs \$3,250 compared with that for a non-automated plant having a typical layout; a plant handling 1,000,000 gallons of ice cream and 250,000 gallons of novelties annually can reduce its labor costs \$33,500.
4. Cottage Cheese, Cream Cheese, and Cultured Milk and Cream. The contractor's report covering layouts and operating criteria for automated and highly mechanized plants manufacturing cottage cheese, cream cheese, and cultured milk and cream shows that a plant receiving 172,000 pounds of milk daily and processing it into a product distribution that is 75 percent small-curd cottage cheese, 10 percent cream cheese, 10 percent cultured milk, and 5 percent cultured cream, can reduce labor costs \$75,500 by using automated equipment and an improved layout.
5. Cheddar Cheese. Although the contractor has submitted his report on this study, its review has not progressed to the stage where significant findings can be summarized.

6. Sweet Cream Butter and Dried Nonfat Milk. Although the contractor has submitted his report on this study, its review has not progressed to the stage where significant findings can be summarized.

### C. Containers

A report is in preparation. It makes a comparative evaluation of three single-service containers and the conventional returnable can for milk served from institutional and commercial dispenser units.

## PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

### A Procedure for Planning the Most Efficient Use of Dairy Manufacturing Plant Resources, Including Labor and Facilities

Glickstein, Aaron; Babb, E. M.; French, C. E.; and Green, J. H., December 1962. Simulation Procedures for Production Control in an Indiana Cheese Plant. Purdue University Agricultural Experiment Station Research Bulletin No. 757.

### Layouts and Operating Criteria for Automated Dairy Product Plants

Tracy, Paul H., March 1963. Layouts and Operating Criteria for Automation of Dairy Plants Processing Milk and Half-and-half. Marketing Research Report No. 568.

Tracy, Paul H., September 1963. Layouts and Operating Criteria for Automation of Dairy Plants Processing Milk, Half-and-half, Cream, Chocolate Drink, and Buttermilk. Marketing Research Report. No. 591.



## COOPERATIVE MARKETING Farmer Cooperative Service

Problem: Farmers continue to expand their use of cooperatives in marketing the products of their farms. In light of the rapid and complex changes taking place in technology and in market organization and practices, research is needed to help farmer cooperatives and other marketing agencies perform needed marketing services both more efficiently and more effectively. Farmer-directors, managers and others, including the public, need more information to assist in making decisions on how cooperatives can maintain and strengthen the bargaining power of farmers, increase efficiency and reduce costs of marketing, and better meet the needs of our mass distribution system for large quantities of products on a specification basis.

Farmer cooperatives are an important part of the distribution system and represent a major potential for meeting farmers' marketing problems in our modern, dynamic system. They are organized and operated to increase farmers' net income. However, cooperatives face many problems in achieving this goal. Cooperatives must find ways to consolidate volume, for example, through internal growth, merger, acquisition or federation, to strengthen their market position and meet the needs of mass merchandising. Ways must be found to reduce costs by increasing efficiency through improved operating methods, better organization and management, and more use of new technologies.

### USDA PROGRAM

The Department conducts a continuing long-range program of basic and applied research and technical assistance on problems of marketing farm products cooperatively. Studies are made on the organization, operation and role of farmer cooperatives in marketing. While most of the research is done directly with cooperatives, the results are generally of benefit to other marketing firms. The work is centered in Washington, D. C. Many of the studies, however, are done in cooperation with various State Experiment Stations, Extension Services, and Departments of Agriculture.

The number of Federal professional man-years devoted to research in this area totals 21.2, of which 1.0 man-years are on the cooperative marketing of citrus, 2.7 to cotton, 3.5 to dairy, 1.0 to deciduous fruit, 0.2 to forestry, 1.9 to grain, 2.6 to livestock, 1.3 to oilseeds and peanuts, 1.0 to potatoes, 2.7 to poultry, 0.2 to rice, 1.0 to sheep and wool, 0.1 to sugar, 1.0 to tobacco, and 1.0 to vegetables.

Research also is conducted under contract with land-grant colleges, universities, cooperatives and private research organizations. During the period of this report, contract research was performed by universities and colleges in Florida, Iowa, Louisiana, Montana, North Carolina, North Dakota and Oregon, and by two private research companies.

In addition, 15 case studies of individual or groups of cooperatives were completed. These were concerned with the improvement of operating methods and the feasibility of coordinating the marketing of two or more cooperatives.

## REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

### Dairy

1. Coordination of marketing. Analysis was completed of the economic feasibility of a coordinated marketing program for dairy cooperatives serving major fluid milk markets in Kentucky and Tennessee. This report analyzes the possibilities inherent in large-area coordination in marketing, and also reveals the methodology required to demonstrate the potential economies and bargaining advantages. The study showed that farmers may obtain substantial savings through a coordinated system for assembling and manufacturing fluid reserve milk. The study also pointed to the need for a pooling system to distribute these savings equitably to all participating dairymen.

A case study was made on the possibility of unifying the operations of two large dairy bargaining cooperatives. The study showed that savings could be made through increased scale of operations, particularly in the manufacture of milk not needed for fluid use. Major gains, however, are expected to be acquired through improved bargaining position and greater market stability. The study also indicated that the greatest stumbling blocks to unification were in the area of non-economic considerations.

2. Pooling and pricing. A report on the pooling status of dairy cooperatives in all Federal order markets was published. This study showed that cooperatives played a key part in the marketing program of Federal order milk pools. In comparison to nonmembers or independent producers, farmer-members of marketing cooperatives far outnumbered nonmembers. They also--through their cooperatives--marketed the major proportion of milk shipped to order markets by performing several vital receiving, processing, transporting, diverting, manufacturing and bargaining functions. Most dairy cooperatives operated in only one Federal order marketing area, although one of



ten associations had expanded marketing operations to include two or more order markets. Three-fourths of the cooperatives serving Federal order markets could be classed as small, having less than 300 members shipping to these markets. Cooperatives have also been instrumental in easing the changeover from can to bulk handling in order markets where, at the time of the study, more than 70 percent of the milk shipped in most regions was bulk tank milk.

3. Merchandising manufactured products - nonfat dry milk. Analysis continued on the study of marketing methods and experiences of cooperatives in selling dry milk. Findings from preliminary analysis indicated that types of nonfat dry milk produced by cooperative manufacturers met users' specifications in sufficient quantities to fulfill demand.

4. Market structure and organization. Highly significant developments in the marketing structure for the procurement, processing and distribution of dairy products have occurred in recent years. To help dairy cooperatives keep abreast of these changes, work was initiated with the North Central Regional Research Committee in a study of adjustments taking place in the structure and organization of the Midwest dairy industry.

5. Improving operating methods and efficiency. A case study was made of the membership dues and marketing fees charged different types of cooperative members by a large sales federation. The study indicated that current marketing conditions call for a change in the relationship between dues and fees to provide equity among members and to develop an efficient sales organization.

A case study made of the operating methods and efficiency of a small milk packaging plant showed that operating problems resulting from declining sales in the existing market may not always be alleviated through the acquisition of routes in a distant market.

A case study was made of a multiproduct cooperative concerning the product mix to best serve farmer-members through its local marketing activities and through the marketing activities of a large sales federation. The study revealed that, in the context of the trends toward diversification of product at the manufacturing level, the dependency of the federation upon one member to provide a certain product requires that that member be able to return to its farmers a price equal to that from production of another product.

A case study made of the feasibility of establishing a small fluid milk bargaining cooperative in a Federal order market served primarily by a large bargaining association indicated that the proposed small cooperative would experience excessive costs in providing a satisfactory service program for its members.

#### PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

##### Dairy

- Davidson, D. R. 1963. Impact of Dairy Cooperatives on Federal Order Milk Markets. FCS General Report 114.
- Davidson, D. R., and Tucker, G. C. Jan. 1963. Benefits Spread by Dairy Co-ops. Article in News for Farmer Cooperatives.



ECONOMICS OF MARKETING  
Marketing Economics Division, ERS

Problem: Within most agricultural processing industries rapid and drastic changes in their market organization and practices are occurring. These changes are affecting both farmers and consumers. Research is needed to keep abreast of such changes and to indicate their probable consequences. There have been substantial advances in recent years in increasing efficiency and reducing costs through adoption of new technology in producing, assembling, processing, and distributing farm products. However, for producers and marketing firms to remain competitive additional information is needed on margins, costs, economies of scale and efficiencies possible in the marketing of farm products. A significant aspect of the problem in marketing is that this type of information must be obtained from firms engaged in business -- in contrast with other types of research where the problem can be transferred to a laboratory, experimental plot, or other simulated situation. Consequently, it requires the cooperation of people engaged in making their living and assisting with marketing economic research on the side, where their own merchandise, facilities, and opportunity for profit and loss to themselves is involved. Another aspect of the problem is that only large firms can afford this type of research, consequently, public research has been requested for the many smaller firms. Furthermore, there is the need for comparison and analysis where even large firms do not have access to the plants and records of competitors.

USDA PROGRAM

The Department has a continuing program to determine the reason for the changes that are taking place in marketing so that ways can be found to increase the efficiency of the marketing system and make it more responsive to changing public needs. Because more than 50% of the consumer's dollar spent for meat products goes for marketing activities, this work encompasses a wide range of subject matter.

It covers all economic aspects of marketing from the time the products leave the farm until they are purchased by ultimate consumers. Much marketing research is functional in nature and could apply to a number of commodities.

A. Market Potentials for New Products and Uses

The Department has a continuing long-term program involving agricultural economists, economists and personnel with dual economic and technical training engaged in research to bridge the gap between laboratory developments and commercial adoption so as to assist producers to realize more rapidly and more fully benefits of lowered costs, increased returns, and expanded markets that new products and new uses can afford. Research is carried on in industrial and food uses at Washington, D.C. and five field offices -- agricultural economists are located at each of the four

Utilization Research and Development Divisions, New Orleans, Louisiana; Albany, California; Philadelphia, Pennsylvania; and Peoria, Illinois; and at the Hawaii Agricultural Experiment Station, Honolulu, Hawaii.

Of the Federal effort involving 20.5 professional man-years, 4.2 are devoted to animal products.

#### B. Merchandising and Promotion

The Department has a continuing long-term program of research in merchandising, management analysis, product distribution, and promotion evaluation, designed to provide useful information to producers, handlers, and distributors by which markets for farm products can be maintained and strengthened.

Merchandising research is conducted to quantitatively measure the impact of selected selling practices and pricing policies on sales of and demand for agricultural products. Research in this area is concerned with specific studies such as: Development of income-expenditure elasticities for selected products; identification of consumer and market profiles; and evaluation of alternative package sizes, displays, pricing techniques, and quality of products on consumer purchases. Along with the merchandising research is a relatively small undertaking involving management type studies designed to improve the efficiency of firms distributing farm products with work at the assembly and wholesale level being emphasized.

Research appraising and analyzing promotional programs of agricultural groups is directed toward studies such as: Organizational structure and procedures of commodity groups for optimum control, coordination, and effective conduct of program; measurement of levels of advertising and promotional intensity necessary to maximize sales; and evaluation of effectiveness of alternative appeals, themes, and techniques in selling farm products.

Of the Federal effort involving 17.6 professional man-years, 1.5 are devoted to dairy products.

#### C. Economics of Product Quality

The Department's program of basic and applied research on the economics of product quality includes study of the problems of seven different commodity groups. Work on all commodities is carried on in Washington. Work on protein content of milk is being done at Davis, California, under contract with the California Agricultural Experiment Station.

Of the Federal effort involving 14.3 professional man-years, 2.0 are devoted to dairy products.



#### D. Marketing Costs, Margins and Efficiency

The Department has a continuing long-term program of research in marketing margins, costs, and efficiency designed primarily to provide useful information on the amounts and trends in marketing margins, costs of marketing, labor and equipment requirements, cost standards, economies of scale, and other factors including marketing practices, affecting costs of marketing through all important trade channels and types of firms and for all farm products marketed in commercial volumes. Most of the research is problem-solving in nature, and is conducted by professional agricultural economists. Some studies are conducted in close cooperation with agricultural engineers and members of other disciplines. In nearly all studies close cooperation is maintained with industry and trade groups and with private firms that generously provide essential data and make plant facilities available for observation and the conduct of various market tests. Although most of the research is conducted by personnel in Washington, D. C., a considerable part of the work is done by USDA professional staff located at field stations in several States. These agricultural economists work closely with State agricultural experiment stations which also share a part of the expense of the cooperative studies.

Of the Federal effort involving 42.2 professional man-years, including cooperative agents paid mainly from Federal funds, 3.5 were devoted to dairy products.

#### E. Market Structure, Practices and Competition

This is a continuing long-term program of economic research to assist farmers and marketing agencies to adapt to changes in market structure, practices and competition. Work in this area is conducted at Washington, D.C., at field offices in Berkeley, Calif., and Denver, Colo., at 20 experiment stations under cooperative agreements or contracts, and by a private firm under contract. The Federal scientific effort devoted to economic research in this area totals 42.4 professional man-years, of which 5.3 is devoted to dairy.

#### F. Information, Outlook and Rural Development

The Department's research program concerning marketing information, outlook, and rural development includes situation and outlook reports concerning prices, costs and margins, employment, marketing services, market structure, means of collecting and disseminating market information, and feasibility of investments in rural areas.

The Department's continuing program of economic research relating to marketing information, outlook, and marketing aspects of rural development is conducted mainly at Washington, D.C.; work on marketing information is

conducted at Baton Rouge, La., Manhattan, Kans., Columbia, Mo., Madison, Wis., and University Park, N.M.; and work on long-term outlook at Berkeley, Calif., and Corvallis, Ore.

Of the Federal effort totalling 13.2 professional man-years, 1.0 is devoted to dairy.

## REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

### A. Market Potentials for New Products and Uses

In view of declining per person consumption of milk, work is underway to assess the market penetration of low-fat (two-percent milk), its impact on total sales, and its potential for increasing consumption. Preliminary results of a survey of milk dealers indicate this type of product has played a significant role in increases here-to-fore reported for skim milk sales. Low-fat milk is apparently more widely retailed than had been supposed. Detailed analyses are underway to define the position the product has attained in a number of markets throughout the country.

### B. Merchandising and Promotion

#### Effect of Different Levels of Promotional Outlay on Sales of Fluid Milk

A study was initiated in March 1963 in cooperation with the American Dairy Association to determine the impact of increased levels of promotional expenditures on sales of fluid milk. The study is designed to determine whether it would be profitable to increase expenditures for promotion of fluid milk above present levels. The levels of expenditures being tested on an annual basis are: 15 cents per capita above present levels and 30 cents per capita above present levels. Present levels of expenditures are being used as a control. The study is being conducted in 6 major market areas and is scheduled to run for a period of 2 years, ending February 1965. In addition to sales data, information is being obtained in each market on level of employment, school enrollments, and selected merchandising practices employed in a sample of retail food stores for fluid milk and ice cream.

### C. Economics of Product Quality

#### 1. Variability of Butterfat Tests

Measurements have been completed and analyses have been made of the day-to-day variability of individual producers' butterfat percentages, the day-to-day variability in the average test of all milk delivered to a plant, and the relationships of "biases" between butterfat percentages from composite samples and those from daily fresh samples during the compositing period, over a wider range of conditions and for longer periods than had been covered in previous studies.



The results present the most complete picture available of the range of variation and the proportion of differences of specified amounts that may be expected to occur when methods of sampling and testing are followed by proficient, unbiased technicians, for several types of comparisons; for example, comparisons between plant averages of 7-, 10-, or 15-day composite tests during the month and averages of fresh tests on from 1 to 4 days during the month, or comparisons between plant averages of fresh tests on 4 days a month and averages of fresh tests on from 1 to 12 days per month.

Multiple regression equations have been developed for estimating the day-to-day variability in the average of fresh tests of all milk delivered to a plant, based on the average within-producer variability and the number of producers, to be used with a seasonal factor for each month. These calculated variances will be the most useful in estimating plant averages for selected days during a month in comparison with the true average for all days during the month if they are based on within-producer estimates calculated (or estimated from two or more daily samples for each producer during the month) for the plant in question instead of being based on the averages shown for all plants. A purely statistical standard cannot be relied on as the sole means of evaluating differences between butterfat percentages from composite tests and from fresh tests. Compositing procedures are very important.

2. Methods of Determining Protein and Solids-Not-Fat as a Basis for Purchasing Milk. Technical work under the AMS portion of the study has been virtually completed. Economic analysis will get underway during the coming year.

#### D. Marketing Costs, Margins and Efficiency

1. Costs and Margins of Fluid Milk Distributors. Net receipts per hundred-weight of milk and cream processed by 80 selected fluid milk distributors declined from an average of \$11.37 in 1961 to \$11.17 in January-March 1962 and \$11.03 in April-June 1962, chiefly because of a shift from retail to wholesale sales and a decline of 7 cents per hundredweight in the cost of raw milk purchased.

2. Efficient Organization of the Southern Dairy Industry. For the Southwest group of States, the actual movement patterns for fluid milk and ice cream were quite different from what they were under programmed equilibrium conditions. The equilibrium movement patterns indicate a need for more specialization in the distribution from processing areas to markets. Projections of demand to 1975 indicate an increase of 35 percent over 1955 with considerable differences among States. The equilibrium conditions for 1975 based on projections of supply and demand indicate somewhat lower wholesale prices for fluid milk than in 1956.

With assembly of milk supplies under optimum conditions, costs could be reduced 10 to 25 percent in different markets, milkshed areas would be reduced in size yet adequate supplies would be available. However, such a change would necessitate considerable action on the part of producers and milk handlers.

A study of long-distance distribution of packaged fluid milk indicated that milk can be distributed at lower costs from local distribution center in the market served than by routes operated from the processing plant at distances over 100 miles from the processing point when the quart-equivalent units delivered are over 1,000 per day.

3. Capacity and Flexibility of Milk Manufacturing Plants. Preliminary investigations seem to indicate that plants with small milk volumes have limited flexibility. Larger plants seem to be more flexible but hesitate to alter the product mix unless price and cost factors appear relatively stable. Nonprice factors, such as availability of specialized labor and alternative markets, also contribute to the stability of the product mix.

4. Cottage Cheese and Frozen Dessert Costs. In 1960-61, costs for processing and marketing cottage cheese averaged 19.7 cents per pound and \$1.15 per gallon for frozen desserts in a sample of Kansas, Missouri, and Oklahoma fluid milk plants. Costs varied considerably among plants for both products. Much of the variation was caused by differences in costs for ingredients and for selling and delivery.

Profitability of handling cottage cheese and frozen desserts varied among the plants. All plants except one received a net profit on cottage cheese, and all except one received a net profit on frozen desserts.

#### E. Market Structure, Practices and Competition

1. Price Wars in City Milk Markets. The study has provided a fairly clear understanding of the causes of retail milk price wars as part of the competitive conditions in city milk markets. It has helped to identify certain functional aspects of some price wars, including: (1) The stimulation of technological innovation; (2) assisting the entry of new firms; and (3) helping to bring outmoded structures of prices into a more realistic relationship to changed conditions. In an effort to retain the viability of competition (including price competition) among dairy firms while mitigating the clearly destructive aspects of over-intense competition and prolonged price wars which have plagued many city markets, recommendations are being prepared with respect to improvements in: (1) Competitive tactics of the individual firm; (2) strategy of the industry of a city market for more orderly revisions of price structures; and (3) improved public policies.

2. Drive-In Dairies in California. Drive-in dairies increased their share of Class I milk sales in Central California from 1.8 percent in January 1957 to 6.4 percent in January 1962. It declined to 5.8 percent by January 1963, with the reduction in the price differential between drive-ins and grocery stores. Major factors contributing to the growth of drive-ins were: (1) The price differential allowed under State minimum price regulations; (2) the efforts of producers to obtain outlets for Class I milk under the California milk classification regulations; and (3) changes in the shopping



habits of consumers. Growth of drive-ins in many other areas of the country is likely to be slower than in California because of differences in the first two of these factors.

3. Structure of the Midwest Dairy Industry. Analysis of background data including trends in geography of milk and cream supplies showed that from 1939 to 1959 density had increased most in and around the most concentrated production area (centering in Wisconsin), though also in Southwestern Missouri and Central Kentucky. Decreases were most pronounced in the Corn Belt and in the Northern Plains States.

Census and Federal milk order data were used to estimate trends in numbers and sales volume of dairymen selling Grade A milk, manufacturing grade milk and farm separated cream. Declines in numbers of dairymen selling these products were especially sharp for farm separated cream and for manufacturing grade milk east of the Mississippi River. Increases in average volume were greatest among Grade A milk sellers.

The pronounced shift in the region from sale of cream to sale of whole milk reflected increased use of whole milk in butter manufacture as well as a decline in the relative importance of butter. Bulk handling has increased sharply throughout the region, contributing to a sharp decline in country receiving stations in the nearby zones of large milksheds.

#### F. Information, Outlook and Rural Development

##### The Effects of an Experimental Milk Price Reporting Service

Prior studies showed that of 543 sellers of milk approximately 53 percent knew the price paid by current buyers. Because of such findings an experimental price reporting service was designed to test two major hypotheses: (1) the price information, which allows a dairy farmer to compute the net paid price of his own and alternative price of buyers or raise his level of knowledge of his own and alternative prices, will make him more price responsive and (2) the increase in dairy farmer's level of knowledge of base prices, fat differentials, and hauling charges will bring about some alteration of competitive practices of the milk-buying firms.

To test these hypotheses a control area and experimental area were established within the same basic Wisconsin milkshed. Price reports were mailed monthly for eight months to farmers in the experimental area. Farmers in both experimental and control areas were interviewed shortly after the last price sheet was mailed. Results showed that farmers who received experimental price reporting services possessed a significantly higher level of knowledge about the base price received for milk in the

previous pay period. They also knew alternative milk prices more often than the control area sellers. However, even though farmers who received experimental reporting service information were better informed there was no immediate mass response to changes in milk prices during the period of study. Few changes in daily milk marketing operations were reported by either wholesalers or farmers who received the price reporting service or those who did not. Furthermore, there was no appreciable change in the range of prices paid for milk during the seven months of the experimental period. The reporting service appeared to do little to increase competition among purchasing firms.

It should be noted, however, that within the experimental area the firm paying the lowest price during the first week of the experiment received several threats of patrons leaving because they had become aware this company was paying a lower price. However, with a slight upward price adjustment this firm maintained its customers; and by the end of the study it stated the information given to its patrons had had no effect on its pricing policy.

#### PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

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CONSUMER PREFERENCE AND QUALITY DISCRIMINATION -  
HOUSEHOLD AND INDUSTRIAL  
Statistical Reporting Service

Problem: With the increasing complexity of marketing channels and methods, it has become almost impossible for the consumer to express to producers either his pleasure or displeasure with available merchandise. In order to market agricultural products more efficiently, we need to understand existing household, institutional, and industrial markets and the reasons behind consumers' decisions to purchase or not to purchase. Information is needed on preferences, levels of information or misinformation, and satisfactions or dislikes of both present and potential consumers. We also need to know consumer attitudes toward the old and new product forms of agricultural commodities and their competitors, and probable trends in the consumption of farm products. We need to know the relationship between agricultural and nonagricultural products and the relationship of one agricultural commodity to another in consumers' patterns of use. Producer and industry groups and marketing agencies consider this information essential in planning programs to maintain and expand markets for agricultural commodities which, in turn, increases returns to growers.

USDA PROGRAM

The Special Surveys Branch of the Standards and Research Division conducts applied research on representative samples of industrial, institutional, or household consumers and potential consumers, in local, regional, or national marketing areas. Such research may be conducted to determine attitudes, preferences, buying practices, and use habits with respect to various agricultural commodities and their specific attributes; the role of competitive products, and acceptance of new or improved products.

The Special Surveys Branch also conducts laboratory and field experiments in sensory discrimination of different qualities of a product. These studies ordinarily relate discrimination to preferences and attitudes as they influence purchases in order to assess the standards of quality, packaging, etc., which are needed to satisfy consumer demands.

In addition to surveys of consumer preferences and discrimination, the Special Surveys Branch also provides consultants and conducts special studies, upon request, for other agencies in the U. S. Department of Agriculture or within the Federal Government, when survey methods can be usefully applied to the evaluation of programs, services, or regulatory procedures of interest to the requesting agencies.

The work of the Branch is carried out in cooperation with other Federal governmental agencies; divisions within the U. S. Department of Agriculture, State Experiment Stations, Departments of Agriculture, and land-grant colleges; and agricultural producer, processor, and distributor groups.

Closely supervised contracts with private research firms are used for nationwide surveys; studies in selected areas are usually conducted by the Washington staff, with the assistance of locally recruited personnel.

The Branch maintains all of its research scientists, who are trained in social psychology and other social sciences, in Washington, D. C., which is headquarters for all of the survey work whether it is conducted under contract or directly by the Branch.

The Federal scientific effort devoted to research in this area during the past year totaled 7.0 professional man-years under regular program funds. An additional 0.1 professional man-year was devoted to research conducted under a transfer of funds arrangement.

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

##### Milk

Field work was completed in June 1963 on a study to provide information about household consumption patterns of fluid milk and insights into the reasons why consumers prefer and/or use one type of fluid product as compared with other types generally available. Interviews were conducted with householders in two cities where low fat fluid milk is presently sold. Questions were asked about such items as past experience with milk, changes in consumption, attitudes toward various types of milk and use of milk away from home and at guest meals. This study represents the consumer phase of a research project being undertaken in cooperation with Economic Research Service to study the market characteristics of a low fat fluid milk of approximately 2 percent butterfat content. Interest in the market potential for this "2 percent milk" has been aroused by a shift in consumer dairy purchases between 1950 and 1962 to fluid and dry products which are low fat or nonfat. During this same period, per capita consumption of all types of fluid milk and cream has dropped 11 percent and farm output of milk has risen, causing a serious problem for the entire dairy industry.



**ECONOMIC AND STATISTICAL ANALYSIS**  
**Economic and Statistical Analysis Division, ERS**

Problem. Because of the instability of the prices he receives and rapidly changing conditions of agricultural production, the farmer stands in special need of accurate appraisals of his economic prospects if he is to plan and carry out his production and marketing activities in an efficient and profitable way. The typical farmer cannot afford to collect and analyze all the statistical and economic information necessary for sound production and marketing decisions. It has long been a goal of the Department to provide the farmer with economic facts and interpretations comparable to those available to business and industry, through a continuous flow of current outlook information; the development of longer range projections of the economic prospects for the principal agricultural commodities; and analyses of the economic implications of existing and proposed programs affecting the principal farm commodities.

**USDA PROGRAM**

The program pertaining to commodity situation and outlook analysis includes the regular publication of 11 commodity outlook reports; holding of the Annual Outlook Conference in Washington in mid-November; participation of commodity specialists at regional or State outlook meetings or at meetings of farm organizations and agricultural industry groups; preparation and publication of special articles bearing on both the short-run and long-run outlook for farm commodities; issuance of comprehensive statistical bulletins containing the principal economic series pertaining to the various commodities; long-range projections of supply of and demand for the major agricultural commodities; and continuing analysis of the impact of existing and proposed alternative farm programs as they affect output, utilization and prices of these commodities.

The total commodity situation and outlook program currently involves 22 professional man-years.

Dairy. This work involves 2.0 professional man-years in Washington. The outlook and situation program provides a continuing appraisal of the current and prospective economic situation of milk and milk products. Appraisals are published 5 times a year in the Dairy Situation, quarterly in the Demand and Price Situation and the National Food Situation, and monthly in the Farm Index. A comprehensive analysis of the dairy situation is presented at the Annual Outlook Conference. Outlook appraisals are frequently presented at regional or State outlook meetings, at meetings of farm organizations, and to various agricultural industry groups. Special analyses are made from time to time on the probable effect of proposed programs on the production-utilization balance of milk and milk products. Basic statistical series are maintained, improved and published for general uses in statistical and economic analysis. A supplement to Dairy Statistics is being prepared for release in the fall of 1963.



### Fats and Oils.

This work involves 2.0 professional man-years in Washington. The outlook and situation program provides a continuing appraisal of the current and prospective economic situation of fats, oils, and oilseeds. These appraisals, developments of interest to the industry, and results of special studies are published 5 times a year in the Fats and Oils Situation, quarterly in the Demand and Price Situation and the National Food Situation, and monthly in the Farm Index. A comprehensive analysis of the fats and oils situation is presented at the Annual Outlook Conference, and more limited appraisals given at meetings with industry groups. Special analyses are prepared from time to time on the probable effect of proposed programs on the price, supply, and consumption of fats and oils and their products. Basic statistical series are maintained, improved and published for general use in statistical and economic analysis. A Statistical Handbook, Oilseeds, Fats and Oils, and Their Products, is being revised for publication in 1964.

Increasing emphasis has been on the effect of increased fat output and export markets on prices and incomes received by farmers.

## REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

### A. Dairy Situation and Outlook Analysis

Due partly to a slower rate of increase in production per cow, total output of milk likely will decline to around 125.3 billion pounds in 1963, from 125.9 billion pounds in 1962. Consumption of dairy products from commercial sources was maintained at year-earlier levels in 1963, but preliminary information from all sources for the first 6 months of the year indicated a 2-percent gain. Nonetheless, output continued above total use as stocks of manufactured dairy products and cream (including butteroil) rose seasonally to a record 15.5 billion pounds, milk equivalent, on June 30, about 0.8 billion pounds above a year earlier.

Considerable time and effort have been devoted to analyzing the effects of existing and proposed Government programs on milk production, marketings, prices, consumption, and stocks. Other work in this area includes an analysis of changes in imports of dairy products, in Federal order markets, and in the regional distribution of milk production and marketings. Long-run projections (5 years, by years) for dairying, developed as a part of a set of ERS projections for the farm economy as a whole, were published in November 1962 (ERS-96). This study indicated that milk production and consumption in 1967 would be about the 1962 level, with Government purchases only slightly below the 1962 record. These projections were revised and extended in 1963. A new series was constructed, beginning with 1950, for sales of whole milk, skim milk items, and cream, and the three categories combined, and published in the June 1963 Dairy Situation.



In the August 1963 Dairy Situation, the implications of cost and price relationships on typical dairy farms of the USDA Cost and Return series were examined. A new estimate was prepared--the cost of adding a cow to the milking herd. Marginal cost estimates were well below price (100 pound basis) for Grade A farms in Central Northeastern United States and in eastern Wisconsin from 1951 to 1962, but on Wisconsin Grade B farms costs were closely in line with price. This latter situation also was true for Central Northeastern farms in 1962 and indicates a relatively unfavorable position for Central Northeastern Grade A farms and Wisconsin Grade B farms. However, the opportunity exists for many Grade B producers to change to Grade A status.

## B. Fats and Oils

Supplies of food fats and oils during the 1962-63 marketing year were a record 16.4 billion pounds (in terms of oil), about 4 percent greater than the year before. Total disappearance rose about 5 percent to a new high, with record exports accounting for most of the increase. The Food for Peace Program again was an important factor in exports. Carry-over stocks of food fats on October 1, 1963 were down slightly from a year earlier, due to a big reduction in soybeans. Stocks of edible vegetable oils, lard and butter were up. Prices received by farmers for 1962 crops of soybeans and cottonseed remained somewhat above CCC support rates whereas flaxseed and peanut prices rested on support. Wholesale prices of all fats and oils during 1962-63 averaged slightly below year earlier levels but oilseed meal prices were somewhat higher.

Most significant development during the year was the strong demand for soybean meal, which far outstripped the demand for soybean oil. The large crush of soybeans resulted in record carryover stocks of about 1.0 billion pounds of soybean oil (crude and refined) on October 1, 1963. Thus, the 1963-64 marketing year started with very large stocks of soybean oil and low stocks of soybean meal. As a result, soybean oil prices were low in relation to prices of both soybeans and soybean meal.

In addition to the regular analytic work and outlook analyses, considerable effort was devoted to improving statistical techniques used in forecasting and the development of new statistical series. Work got underway on a comprehensive statistical bulletin for fats, oils, oilseeds and their products. This handbook is badly needed by commodity analysts, as it has been 10 years since the issuance of the last comprehensive compilation of data. Greater attention is being given to some of the minor oilseed crops and our expanding foreign markets. Long-run projections (5 years) were developed for the major oilseeds, fats and oils as part of an overall set of ERS projections for the farm economy.

## PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Dairy

- Cummins, David. August 1963. Implications of cost and price relationships on typical dairy farms. Dairy Situation.
- Mathis, A. G. March 1963. A changing calendar for milk production. Agricultural Situation.
- Mathis, A. G. Dairy Situation. Published 5 times a year. ERS, USDA, Washington, D. C.
- Miller, Robert H. February 1963. Utilization of dairy surpluses. Dairy Situation.
- Miller, Robert H. April 1963. Payments over minimum prices in Federal order markets. Dairy Situation.
- Special Study. June 1963. Sales of fluid milk products. Dairy Situation.
- Special Study. August 1963. Patterns in milk utilization. Dairy Situation.





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A Summary of Current Program and  
Preliminary Report of Progress

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SUPPLEMENT

for

DAIRY RESEARCH 1/

of the

UNITED STATES DEPARTMENT OF AGRICULTURE

and Cooperating Agencies

containing:

Infectious and Non-infectious Diseases  
of Cattle

Foot-and-mouth and Other Exotic Diseases  
of Cattle

Parasites and Parasitic Diseases of Cattle

UNITED STATES DEPARTMENT OF AGRICULTURE

Washington, D. C.

December 31, 1963

1/ The research work on diseases and parasites of cattle reported  
in this supplement is the same as that included in the Livestock  
Report. This supplement should be treated as part of the Dairy  
Report.

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INFECTIOUS AND NON-INFECTIOUS DISEASES OF CATTLE  
Animal Disease and Parasite Research Division, ARS

Problem. Losses from infectious and non-infectious diseases of cattle, other than those due to parasites, are estimated at approximately \$600 million annually. These losses materially increase costs of production and conversely decrease profits. In turn, they contribute to the cost of every purchase of meat, milk, and other cattle products to the consumer. Some of these diseases are transmissible to man. Determination and definition of the causes of cattle diseases, explorations for efficient methods of diagnosis, prevention, control, and when feasible, eradication, are the purposes of the research program.

USDA PROGRAM

The Department has a continuing long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of cattle. Research is being conducted on the diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 50.7 professional man-years. This effort is divided among sub-headings as follows:

Brucellosis, 2.3 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the universities of Maryland, Minnesota, and Wisconsin.

Paratuberculosis (Johne's Disease), 4.0 at the National Animal Disease Laboratory, Ames, Iowa.

Vibriosis, 5.1 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreement with the New York State Veterinary College.

Tuberculosis, 4.6 at the National Animal Disease Laboratory, Ames, Iowa, and through contract with the Michigan State University.

Mucosal-Respiratory Disease-Complex, 1.1 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Indiana and Iowa Experiment Stations (mucosal) and the Colorado State University (respiratory - rhinotracheitis).

Mastitis, 6.2 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California.

Respiratory Disease (Shipping Fever), 5.0 at the National Animal Disease Laboratory, Ames, Iowa.



Leptospirosis, 4.0 at the National Animal Disease Laboratory, Ames, Iowa.

Infertility, 3.0 other than vibriosis and trichomoniasis, at the National Animal Disease Laboratory, Ames, Iowa.

Epizootic Bovine Abortion, 3.4 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California.

Enteric Infections, 4.0 at the National Animal Disease Laboratory, Ames, Iowa.

Leukosis, 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

Foot Rot, 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

Keratitis, 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

#### RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 73.2 professional man-years divided among subheadings as follows: Brucellosis 5.8, vibriosis 6.4, tuberculosis 0.8, mucosal-respiratory disease complex 6.2, mastitis 10, respiratory disease (shipping fever) 4.7, leptospirosis 7, infertility other than vibriosis and trichomoniasis 5.2, epizootic abortion 0.5, enteric infections 2.5, foot rot 0.8, infectious keratitis 2.1, eurolithiasis 1.3, other diseases (clostridia, pulmonary emphysema, white muscle disease, etc.) 17.8. Colorado, Connecticut, Georgia, Kansas, Maryland, Michigan, Minnesota, Ohio, Virginia, and Wisconsin are conducting studies on brucellosis. Seven southern and three northeastern states are working on vibriosis through two regional research projects (S-30, Diseases of Reproduction and NE-40, Pathology of Breeding Failure). Wisconsin and Michigan are conducting research on improving present tests for greater sensitivity in diagnosis of tuberculosis. Eight north central states are conducting research under NC-34 Mucosal Disease. Florida coordinates related work on an informal basis with the north central states on mucosal-respiratory disease complex. North central, northeastern, southern, and western regions are all conducting research on mastitis. Seven north central states and the Department are cooperating through regional research (NC-34, Shipping Fever of Cattle). Five southern and two northeastern states cooperate in regional research (S-30, Diseases of Reproduction, and NE-40, Pathology of Breeding Failure). Cooperative regional studies among four northeastern states (NE-40, Pathology of Breeding Failure) and four southern states (S-30, Diseases of Reproduction) are being made on infertility, other than vibriosis and trichomoniasis. California, in cooperation with the Department, is conducting research on epizootic abortion. Arizona, Connecticut, Colorado, and Missouri are studying bacteria and viruses found associated with intestinal infections of cattle, known as enteric infections. Colorado is conducting research on foot rot. Arizona, Kansas, Montana, Nebraska, Oklahoma, and Texas are conducting research on infectious keratitis. Five western states are cooperating in regional research (W-41, Urinary Calculi of Beef Cattle)- Urolithiasis. All regions are conducting some research on Other Diseases (clostridia, pulmonary emphysema, white muscle disease, etc.)

Industry and other organizations are engaged in the preparation of marketable biologic and pharmaceutical products. They conduct experimentation on vaccines and the formulation of chemical compounds and other medicinal substances for prevention and treatment of diseases of cattle. These companies generally will utilize their own facilities. Information gained in their research generally is confidential in nature as are expenditures for research and development. It is estimated that 80 professional man-years are devoted to this work by industry and other organizations.

## REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

### A. Brucellosis

In 1961, at the Animal Disease Station, Beltsville, Maryland, the persistence and localization of Brucella abortus infection in 169 bovine females was determined. Major sites of localization were the udder and supermammary lymph glands. Infection was shown to persist up to 11 years in these locations.

After exposure to virulent Brucella abortus strain 2308, cattle produced two types of sero-agglutinins. These could be differentiated on their ability to withstand heat (65 C for 15 minutes). Subsequently the relative percentages and the persistence of the two types of seroagglutinins differed between the resistant cattle and the cattle that became infected. In general, resistant cattle produced a small percentage of heat stable sero-agglutinins which were limited to the first 70 days after exposure. However, cattle that became infected produced predominantly heat stable sero-agglutinins throughout the course of the disease.

Acidified plate test antigens were shown to be incapable of differentiating serologic reactions of infected cattle from those of Brucella resistant cattle. The tests were not selective but rather the results depended upon the magnitude of the titer to the standard sero-agglutination test and the pH of the antigen employed.

An experimental complement fixation test was less effective than the heat inactivation test or the standard sero-agglutination tests for the detection of infection in the immediate post-exposure period, except in a few animals that became infected but did not abort.

The potential value of infra-red spectroscopy as means of identifying and classifying typical and atypical strains of the genus Brucella was investigated further.

Five hundred and seventy-five apparently normal fetuses, aseptically obtained, were studied from a bacteriological point of view. Serological tests, direct cultures on several media, as well as inoculation into embryonating eggs and guinea pigs, showed that bacteria were generally present in embryos and that the fetal skin seemed to harbor more microorganisms than other tissues and organs in the fetus.



Brucella isolations were made from the fetuses of negative cows and from some having titers of 1:2000 and greater. Fetuses of every age group harbored Brucella, and organisms were isolated from about 4 per cent of all fetuses examined. More than half of the isolations were made from the skin. No Leptospira were isolated at any time.

Immunizing agents have been studied in an attempt to develop a satisfactory vaccine for adult cattle and over-age calves. Strains of Brucella of reduced infectivity for laboratory animals have been produced using (a) streptomycin-dependent strains, adapted to living on 1000<sub>u</sub> per ml. for 1 year and then adapted to growing without the antibiotic, and (b) embryonating egg-adapted strains. The latter have been serially passed through embryonating eggs from 100 to 500 times, and are highly potent in eggs. A very mild disease is produced in laboratory animals, but following challenge they exhibited a high degree of resistance to infection with virulent forms of the organism.

In 1962, at the National Animal Disease Laboratory (NADL), Ames, Iowa, experiments were completed to determine immunogenic response of calves vaccinated at different ages with Brucella abortus Strain 19. Midway through their first gestation 69 vaccinated cattle and 22 nonvaccinated controls were exposed conjunctivally to approximately  $7 \times 10^5$  cells of virulent Brucella abortus Strain 2308. Proof of infection was based upon isolation of the organism from one or more of the following sources: milk, dam's blood, uterine contents and fetus at parturition, or tissues obtained at necropsy.

Eight of 24 calves vaccinated at 4 months of age became infected and 5 aborted. Eight of 22 calves vaccinated at 6 months of age became infected and 5 aborted. Seven of 23 calves vaccinated at 8 months of age became infected and 6 aborted. Twenty of 22 nonvaccinated controls became infected and 15 aborted.

Although no significant differences in the degree of vaccinal immunity could be shown among the three groups of cattle vaccinated at different ages, vaccination at 4 months of age materially reduced the problem of persistent postvaccinal titers.

Natural infection with Brucella abortus in 2 bulls was studied over a period of several years. Serologic, bacteriologic, and histopathologic examinations were correlated with the clinical manifestations of the disease.

Seroagglutinin and semen plasma agglutinin titers persisted for at least 5 years. Brucella abortus was consistently isolated from the semen of both bulls throughout the course of the disease.

At necropsy Brucella abortus was isolated from the testes, epididymides, seminal vesicles and the ampullae of the ductus deferens.

Pathologic changes occurred throughout the genital tract. Granulomas, including sperm granulomas, were found in the epididymis of one bull.

The high percentage of ejaculates containing viable Brucella abortus emphasized the need for frequent bacteriologic examinations of semen for evidence of Brucella abortus infection in all bulls used for artificial insemination or natural service.

A modified Coombs' test for the detection of incomplete antibodies was evaluated on serum frozen and stored at dry ice temperature from a previous exposure of vaccinated and nonvaccinated cattle. The test failed to reveal the presence of any incomplete antibodies during the course of the disease.

The conglutinin-complement absorption test was also evaluated on the same lots of frozen and stored serum. Under the conditions and limitations of the experiment the conglutinin-complement absorption test appeared to have no advantage over the standard seroagglutination tube and plate tests or supplementary tests (heat inactivation, complement-fixation, or acidified plate antigens). Furthermore, the complexity of the test rendered it less adaptable for a routine diagnostic laboratory tool.

The entire stock culture collection was repacked and recataloged for the move to the new Ames laboratory, consequently all lyophilized cultures are more readily accessible for future research purposes.

A critical study of the tube agglutination test for the diagnosis of brucellosis in cattle has been inaugurated within the last few months. Sufficient data has not been obtained on any of the variable factors to submit results at this time, but considerable information has been accumulated. Perhaps a better testing procedure may not result from this investigation, but it is possible that some minor changes could be made, which might improve the test.

An attempt has been made to develop a vaccine satisfactory for adult animals and over-age calves, which will not produce a lasting titer. Streptomycin-adapted strains, grown with 20,000 micrograms per milliliter of the antibiotic over a period of time, have been used for guinea pig vaccination. These strains are not streptomycin-dependent, but have been modified in growing with the antibiotic to such an extent that vaccinal titers produced by them are low and transient.

Six embryonating egg-adapted strains of Brucella are being serially carried forward in eggs, one strain having been passed 626 times and others through a lesser number of transfers. Results of trials with egg-adapted Strain 19 (175th passage) and B.C. (600th passage) indicate that both strains have some value as immunizing agents.

Experimental work on the protective quality of cattle blood serum has been confined to further study of 106 vaccinated heifers, which have been tested at monthly intervals.



In 1961, at the University of Maryland under a cooperative agreement with the USDA, studies on Actomer were continued regardless of the toxic effect of the acetone in which it must be dissolved. Experiments, using Actomer in guinea pigs have shown the drug to be effective for Brucella, even in very dilute solutions.

In 1962, Actomer was further studied in relation to its solubility in non-toxic agents. Mice have been used for the in vivo experiments.

In 1961, at the University of Minnesota under a cooperative agreement with the USDA, the experimental work on the protective qualities of cattle blood serum against Brucella was confined to a study of the serum obtained from calves and heifers. Data has been accumulated to complete the results on more than 2000 animal sera.

A Brucella agglutinating component (11.7 S) has been isolated from the milk of 2 cows and appears to differ in several major respects from the brucella agglutinating macroglobulin (19 S) of blood serum.

Epidemiologic, serologic, and bacteriologic studies of Brucellosis "Problem Herds" have been of material assistance in establishing 7 brucellosis-free counties in Minnesota during the past 8 months.

A differential diagnostic test was developed which may be useful in differentiating "non-specific" and "specific" agglutinins for brucella in blood serum and this new test is now undergoing further evaluation.

In 200 herds with long histories of brucella infection, the plate sero-agglutination test failed to identify almost 60 percent of probably-infected cows. In 681 herds, without such a long-standing history of infection, the plate test failed to identify only 16 percent of probably-infected animals. In the problem herds, the combination of plate, tube and whey agglutination tests properly identified 40 of 46 animals proved bacteriologically to be infected.

Herds with consistently positive ring tests which fail to disclose reactors to the plate sero-agglutination test have also been investigated. In most cases, a single animal has been found to be causing the ring test reaction. All were identified by the combination of plate, tube and whey tests.

In 1962, at the University of Minnesota under a cooperative agreement, studies of nonspecific agglutinins (NSBA) in bovine milk were continued to develop improved methods of isolation and increase the yield for further characterization of the macroglobulins. Studies on the use of mercaptoethanol to detect macroglobulins (NSBA) in bovine milk were inaugurated using the procedures which were developed last year.

Studies on mercaptoethanol to detect macroglobulins (NSBA) in blood serum of cattle, which react to the tube sero-agglutination test at dilutions of 1:25 or greater, are being continued in the development of a new procedure which may be useful in the field.

A Modified Cream Ring Test has been developed as a result of studies during 1960-61 which showed that the present cream ring test may fail to detect brucella agglutinins in up to 50% of cream samples tested when such cream has partially deteriorated or soured prior to sampling. The new Modified Cream Ring Test is being evaluated in this laboratory and five field laboratories by comparison with epidemiologic, serologic, and cultural studies of animals in cream ring test positive and negative herds. Results to date appear to support the developmental work in which it was observed that the Modified Cream Ring test would detect up to 50% additional samples containing brucella agglutinins in those cream samples which were partially deteriorated or sour.

Studies of problem herds are continuing using nine serological tests of serum and milk supplemented by bacteriologic and fluorescent antibody studies to isolate or identify the organism from those animals which are slaughtered. With the aid of these procedures 20 counties in Minnesota have been certified as brucellosis-free, and the studies are progressing in approximately 50 other counties. It has been interesting to note that in the first 18 counties certified - some for several years - only 5 herds have been found to have reinfection.

Studies of the milk brucella ring test are continuing to obtain more information regarding optimum methods of storage, handling, and preserving composite milk samples collected for Babcock testing in the plants in order that optimum samples for BRT testing may be obtained from these composite samples. Studies are now in progress to determine the effect of different concentrations of mercuric chloride on brucella agglutinins in the milk of cows at an earlier stage of infection. Studies are being conducted using 1½ ml. and 2 ml. of milk along with the regular milk Brucella ring test. These are being accompanied by studies of herd size and the effect of the number of animals contributing to the pool of milk on the present sensitivity of the brucella ring test under Minnesota conditions. These studies are particularly pertinent since the size of Minnesota herds is increasing each year.

In 1961, the University of Wisconsin, under a cooperative agreement with the USDA, developed a culture system using guinea pig monocytes for the assay of toxicity and antigenicity of extracts isolated from cells of B. abortus. The basic characteristics of a strain of brucella phage, useful in taxonomic work, have been described.

In 1962, at the University of Wisconsin, in studies of 262 herds with long standing infection, 230 were freed of brucellosis by a combination of serologic tests. In these herds the combination of plate tube and whey agglutination tests properly identified 41 of 48 shedders. The tube test alone identified 40 and the plate test alone only 25. The discrepancy between plate and tube test results on infected animals could be reduced by half but not eliminated by incubating plate tests for 15 minutes.



Two hundred forty-nine herds with consistently positive ring tests but without reactors to the plate test have been studied. In most cases, a single animal has been shown by titration with pooled negative milk in a ring test, to be responsible. Of 49 such animals which were subjected to bacteriologic examination, 18 were shedders. All were properly identified by the combined whey and tube tests.

Fractions of Brucella abortus have been studied in a culture system, using guinea pig monocytes. Differences have been found in toxic and hypersensitive effects of various fractions.

A phenotypic morphological effect of brucellaphage on some strains of B. abortus has been shown to be due to a carrier state.

#### B. Paratuberculosis (Johne's Disease)

In 1961 research was continued at the Animal Disease and Parasite Research Division's Station at Auburn, Alabama, on a herd of cattle infected with Johne's disease which had been under study for 56 months. During this period, 94 animals have been culled and examined post-mortem. Neither the complement-fixation test nor the hemagglutination test can reliably distinguish between infected animals and non-infected animals as determined by postmortem results. Although the skin test is commonly used as a diagnostic test, results thus far obtained in this herd indicate that it is far from satisfactory.

An experiment designed to study the use of a vaccine against Johne's disease in sheep has been in progress 4 years. Postmortem examinations have been conducted on 25 vaccinates and 31 controls. A few typical small acid-fast bacilli were observed in the intestinal mucosa of 2 vaccinates and 1 vaccinate was destroyed in an advanced stage of the disease. Two controls died from Johne's disease, 8 others were destroyed in advanced stages of the disease, and 2 animals randomly selected were found to be moderately infected on post-mortem. From these results it appears that the vaccine has definite value as a control measure for Johne's disease.

Four rabbits were inoculated intravenously with Mycobacterium paratuberculosis obtained by digesting infected intestinal mucosa with trypsin. These rabbits all remained healthy and in 3 instances typical small acid-fast bacilli were observed in smears prepared from intestinal mucosa. However, no evidence of progressive Johne's disease was observed in any of the rabbits.

Primary cultivation of M. paratuberculosis from infected tissues was best accomplished by trypsin digestion of intestinal mucosa, followed by treatment with NaOH, and inoculation of lymph gland-egg yolk medium. It was found that the retarded growth exhibited by organisms from infected lymph glands might have been due to antibodies present in the lymph gland tissue. Growth of a recently isolated strain of M. paratuberculosis was not inhibited by 10,000 units of penicillin when the organisms were seeded on lymph gland-egg yolk medium, but there was no growth on lymph gland medium.

Protoplasm from M. paratuberculosis was used as an antigen for antibody production and as an antigen in precipitin tests. When it was used to elicit antibodies it produced a nodule at the site of inoculation and the animal produced precipitating antibodies within 2 weeks, but it did not react to intradermic johnin. When the protoplasm was used as a precipitin antigen, it reacted strongly with the serum from a sheep with clinical Johne's disease, and weakly with sera from sheep vaccinated with whole M. paratuberculosis or exposed to infection with Johne's disease but not showing clinical symptoms.

In 1962 the work on paratuberculosis was moved to the National Animal Disease Laboratory, Ames, Iowa. The herd of cattle ranging in numbers from 161-195 animals in which Johne's disease has been a severe economic problem has been under study for 68 months. During this period, 119 animals have been culled and examined postmortem. One of the 25 that were culled during the past 12 months showed clinical signs of Johne's disease. Neither the complement-fixation test nor the hemagglutination test can reliably distinguish between infected animals and noninfected animals as determined by postmortem results because M. paratuberculosis were found in 25% of the cattle negative to the complement-fixation test and in 36% of the cattle negative to the hemmagglutination test. The skin test using intradermic johnin is commonly used as a diagnostic test, however, results thus far obtained with intradermic johnin in this herd indicates that it needs much improvement because M. paratuberculosis were found in 40% of the non-reactors examined postmortem.

Two experimental skin test products were also tried on this herd. One was almost equal to standard johnin in potency and the other was about 2/3 as potent.

Acid-fast bacilli were demonstrated in the lungs, spleens, kidneys and livers of rabbits for at least eight weeks after they had been injected intravenously with either killed or live M. paratuberculosis. Therefore, it is questionable whether a true infection was established in rabbits with the live microorganisms.

Continuing studies of various types of media showed that lymph node-egg yolk medium is better than simple egg yolk or lymph node media for primary isolation of M. paratuberculosis. A medium prepared from infected intestinal mucosa produced luxuriant growth of M. paratuberculosis after prolonged incubation.

Rabbits and guinea pigs were vaccinated with a protoplasmic antigen and the controls with heat-killed organisms. They were skin tested with johnin and tuberculin. The skin reactions due to protoplasmic vaccine were small or absent. Those due to vaccination with heat-killed organisms were very large. Gel-precipitin tests with sera from protoplasm vaccinated rabbits produced only one definite precipitation zone. No precipitation zones were produced by sera from rabbits vaccinated with heat-killed organisms.



A new proteolytic enzyme (X-108) prepared by American Cyanamid Company, was found to be better than trypsin for digestion of tissues infected with Mycobacterium paratuberculosis and liberation of the bacilli.

### C. Vibriosis

In 1961 the studies on vibriosis were in progress at the Animal Disease Station in Beltsville, Maryland. The morphology and biochemistry of bovine Vibrio strains isolated from 87 cattle in a survey of 56 herds were studied. The V. fetus strains (pathogenic) were separated into 2 major types and a subtype of Type 1, by colony dissociation and biochemical reactions. Type 1 strains grew fastidiously and growth was enhanced by the addition of glutathione and sodium thioglycollate to the medium. Both smooth and smooth-cut glass colonies were observed in primary cultures. Type 2 was characterized by more abundant growth and numerous stable variant colonies. Both smooth and rough colonies were observed in primary cultures. Subtype 1 strains grew moderately in culture mediums. Cut glass colonies were observed only at primary isolation. Vibrio bubulus strains (nonpathogenic) grew well on blood agar but they did not adapt easily to Albimi agar. Smooth and granular colonies were observed in primary cultures. Biochemical tests which were made on cultures of smooth and variant colonial forms failed to show any change in activity due to dissociation.

Bulls that were infected with Type 1 and Subtype 1 strains of V. fetus infected all heifers bred to them. The infected heifers became repeat breeders. However, bulls that were infected with Type 2 V. fetus infected only 1 of 12 heifers by breeding, 11 of which required only 1.4 services per pregnancy. Vibrio which was biochemically similar to the Type 2 exposure strain was isolated from both the vagina and the rectum of 1 heifer. This heifer was apparently not pregnant at slaughter 21 days after service.

A bull that was infected with Type 1 V. fetus infected 12 heifers at their first service. Uterine infection was found in 9 of these heifers at necropsy. A neutrophilic and lymphocytic infiltration of the endometrium was found on histopathologic examination. In contrast, neither infection nor histopathologic changes were observed in 5 virgin heifers that were necropsied at intervals during the estrus cycle. Seven heifers that were bred to a non-infected bull became pregnant, but all were free of histopathologic changes at necropsy.

Cultures of the three types of V. fetus and fecal samples from cows infected with Type 2 V. fetus, were given per orum to noninfected cows. Isolations of Type 2 V. fetus were made from 21 of 60 post-exposure fecal samples. At necropsy, Type 2 V. fetus was isolated from the intestinal tract from 3 of the 7 animals and also from the bile of one cow.

In 1962, most of the work on vibriosis was done at the National Animal Disease Laboratory at Ames, Iowa. Comparative infectivity studies of 20 strains of V. fetus included 12 strains of type 1, 5 strains of subtype 1, and 3 strains of type 2. Only type 2 strains consistently infected the gall bladder and

duodenum of mice, guinea pigs, and rabbits. These results compare favorably with experiments in cattle in which only type 2 V. fetus was recovered from the gall bladder and/or duodenum. It was concluded, therefore, that the digestive organs may harbor virulent V. fetus intestinalis and remain the source of infection in cases of sporadic abortion in cattle.

Chemical, serological, and toxicological analyses were made on cell wall, intracellular, and extracellular fractions of the organism. The cell wall is composed of a complex group of amino acids characteristic of Gram negative organisms. At least 3 antigen-antibody complexes were found in agar-gel diffusion tests. Only the intracellular fraction was lethal for mice in the preliminary studies made.

A liquid culture medium was developed in cooperation with the chemical and physical investigations which improved the yield of V. fetus cells from 0.5 gram to 2.0 grams per liter of culture medium.

In 1961, at the New York State Veterinary College, Ithaca, New York, under a cooperative agreement with the USDA, experiments were conducted to determine if vibriosis was spread by the methods commonly used for semen collection in an artificial insemination stud. Thirty-one bulls were used - half were collected in the usual manner and half with a so-called strict collection technic with no false mounts. Over a 2-year period, no new cases of vibriosis have been detected in the strict collection group, whereas 2 new cases were observed in the regular group. The strict collection method appears to minimize the chances of spread of the infection.

Eight bulls were treated by the application of a 1% furoxone ointment to the penis and sheath. Ten bulls were treated with the same ointment plus a 1% furaltadone solution in a series of three treatments at 47-72 hour intervals. Seven bulls, following treatment, were found to be free of V. fetus at the end of the experiment. The procedures are being continued using the same chemicals in a more absorbable base.

Attempts to find more suitable culture media and technics are being continued. Albamycin has proved highly useful to suppress the growth of B. proteus. The filtration technic of Plummer and associates is being used regularly with highly encouraging results. In the filtered material only 2 percent of the cultures were overgrown, whereas 73 percent were overgrown when non-filtered material was used.

#### D. Tuberculosis

In 1961, research workers at the ADP Station at Auburn, Alabama, reported finding a herd consisting of 200 cattle, all thought to be free of Johne's disease and tuberculosis, disclosed 24 animals with reactions to tuberculin and/or johnin. Three of these were calves. The 21 adults were slaughtered, examined post-mortem for tuberculosis (none was found) and material was obtained from each animal which was processed and inoculated onto medium and



into small laboratory animals. None of the laboratory animals developed lesions of tuberculosis or reacted to the tuberculin test. However, acid-fast bacilli were obtained from cultures from 3 animals. These have not been identified. The ileocecal valves and adjacent regions of the intestinal tract were examined for the presence of bacilli resembling Mycobacterium paratuberculosis. Such organisms were found in the intestines of 3 animals. Material from these three specimens has also been cultured, but this work has not yet been completed. Titers of 1/10 or more were observed in serums of 15 animals prior to skin testing and this titer remained fairly constant each week for four weeks.

In 1962, at the National Animal Disease Laboratory, at Ames, Iowa, data were collected on the response in cattle to intradermal cervical tests with different strengths of tuberculin given in 0.1 ml. amounts. The NADL herd was tested on the assumption that the animals were free of tuberculosis based on a history of no tuberculosis in the herd for over 25 years.

The skin reactions were recorded in terms of increases over and above the original skin thickness. The reactions ranged from (pp) a pin point disturbance through (p<sup>1</sup>) 2 mm. increase, (p<sup>2</sup>) 3 mm. increase, (p<sup>3</sup>) 4-5 mm. increase, and (p<sup>4</sup>) 5-6 mm. increase. In general, a p<sup>2</sup> or greater is considered a significant reaction.

The data showed that when the animals were tested with ARS tuberculin diluted to 10% of the regular strength supplied for use in the field, no animals had greater than pp reactions at 48 hours and only 3 of 120 animals had p<sup>1</sup> responses at 72 hours. In contrast, testing the same animals with regular strength tuberculin resulted in 14 p<sup>1</sup> and 3 p<sup>2</sup> reactions at 48 hours. Ten p<sup>1</sup> and 3 p<sup>2</sup> reactions were found at 72 hours. Testing 69 of the animals with tuberculin concentrated to 4 times the regular strength did not give results differing greatly from those occurring with regular tuberculin, although 1 p<sup>3</sup> and 1 p<sup>4</sup> reaction was observed at 48 hours and 2 p<sup>3</sup> reactions at 72 hours.

The evidence indicates that a less concentrated tuberculin results in fewer skin reactions of all sizes in a herd apparently free of tuberculosis.

As part of a cooperative research problem with Chemical and Physical Investigations, a comparison of the antigens in BCG culture filtrate, water extracts of BCG, and preparations from unextracted and extracted BCG subsequently broken under mechanical pressure, have been compared. By gel-precipitin techniques, common antigens were demonstrated in all of the preparations. The cracked cell preparations had an additional precipitin line not demonstrated with the other antigens.

In 1961 and 1962, at Michigan State University, East Lansing, under a contract with the USDA, investigations were continued on the cause or causes of no-gross-lesion tuberculin reactors and to improve methods of diagnosis of bovine

tuberculosis. All acid-fast microorganisms isolated by various methods from animal tissues, including skin lesions, and soil samples were identified insofar as possible by selected morphologic, cytochemical, infectivity and sensitizing characteristics. Emphasis was placed upon there being no single test which conclusively identifies any one species of the known mycobacteria. For instance, *Mycobacterium tuberculosis* produces nicotinic acid and this is perhaps the most dependable and widely used characteristic for confirmation. However, *M. microti* and *M. ulcerans* are also positive. The demonstration of the tubercle bacilli in pathological material is the only sure method for diagnosis.

A wide spectrum of acid-fast organisms, including many atypicals with varying degrees of virulence and all reacting to some extent to mammalian, avian and to various purified protein derivatives (PPD), were found in the tissues of tubercular positive cattle. To date mycobacteria isolated from 263 cases have been tentatively identified. Of these, 81 were from bovine tissues, other than skin lesions, 22 being *M. bovis* and 2 *M. avian*; 85 from skin lesions, 5 from bovine semen specimens which may have become contaminated at time of collection, 96 from swine lymph nodes, 1 being *M. bovis*, 35 from soil and barn samples, 5 from feed samples and 1 each of guppy and mink origin. In some instances a number of isolants from a single case were tested.

The isolation of many acid-fast organisms, which may be incorrectly called "atypicals", from tuberculin positive cattle that are infective to laboratory animals reveals for the first time a new group of organisms that must be fully evaluated by large animal studies. Infectivity, sensitivity, transmissibility and serologic and immunologic behaviors must be investigated before these organisms can be cataloged and their importance in disease control evaluated.

Research findings that the procedures used for the isolation of acid-fast organisms from samples containing low populations is applicable for the isolations from samples containing high populations. However, the reverse is not true. A total of 121 cases consisting of tissue samples were each processed by two methods, the results reported show there were 22 positives isolated by the sodium hydroxide method and 44 isolates by the pentane-digest method.

The difficulties encountered in the classification of acid-fast organisms isolated from gross lesion and no-gross-lesion reactors and the increasing interest in the pathogenic significance of unclassified acid-fast bacteria have led to the initiation of research concerned with the nature of proteins in and produced by various types of acid-fast organisms. The primary purpose of current studies is to develop procedures to produce more specific sensitins for the identification and differentiation of the various mycobacteria and mycobacterial infections in animals.



### E. Mucosal-Respiratory Disease-Complex.

In 1961 the studies conducted at Purdue University, Lafayette, Indiana, under a cooperative agreement with the USDA on reciprocal cross protection tests in calves indicated the Indiana virus diarrhea agent (IVD), the Oregon virus diarrhea agent (OVD), and the Indiana mucosal disease agent (IMD) were immunologically identical. Attempts were made to neutralize the cytopathogenic OVD agent with 14 different types of antiserum. Seven types related to the mucosal-disease virus-diarrhea complex did neutralize this agent the others did not. Various determinations were made of the properties of the OVD agent. It was found that it contained little or no lipid, was between 50 and 100 millimicrons in size, did not cause hemadsorption of guinea pig erythrocytes, and was destroyed by 56 C. in approximately 30 minutes.

The OVD agent did not readily adapt to embryonated eggs, suckling mice, or rats. Sheep fetuses propagated the agent in low concentrations following in utero inoculation.

A specific pathogen free cattle herd was originated by performing 22 caesarean sections and raising the calves in an isolated environment.

Other results indicated that several of the mucosal disease and virus diarrhea agents are related. This provides evidence that these agents are actually part of the etiology of the diseases and indicates that virus diarrhea and mucosal disease may be different clinical manifestations of the same disease. This hypothesis will be difficult to prove until means are devised to produce the typical clinical syndromes.

Calves contact-exposed to inoculated sheep developed signs of disease. When these calves were challenged with virulent blood they developed typical signs of experimental virus diarrhea. Blood collected from the sheep during the period of leukopenia was infective for calves, indicating that viremia existed at the time sheep-calf contact was made. Calves infected with the sheep blood resisted challenge with virulent bovine blood.

Attempts to demonstrate calf-to-calf contact transmission were unsuccessful. Calves exposed to virus by the nasal-oral route did not develop signs of disease. When these calves, and calves in contact exposure with them, were challenged with bovine virus they all reacted in a typical manner. The contact calf exposed to an intravenously inoculated pen-mate did not develop signs of disease. It was fully susceptible when later challenged.

In 1962, at Purdue University under a cooperative agreement, investigations of field outbreaks in the mucosal disease complex continue to reveal a sporadic distribution of both MD and VD. Chronic diarrheal syndromes in feeder calves continue to pose a diagnostic problem for practicing veterinarians in Indiana.

There is usually no history of an acute disease syndrome (such as acute virus diarrhea) in these chronically affected herds. Invariably only a portion of the herd is chronically affected. It is probable that affected herds experienced a previous, mild infection of short duration, although this information is not always obtained from the herd history. In these cases, diagnosis of VD-MD complex is made on a basis of history (when available), clinical observation, and serology. Serological diagnosis is based on high titers of neutralizing antibody against the type strain of VD virus--Oregon C24v.

In three trials, intravenously inoculated sheep were placed in direct pen-contact with susceptible calves. Control transmission tests with calves were performed in which orally and parenterally inoculated calves were placed in direct contact with other susceptible calves. Bovine blood was used as stock virus. In no case was contact infection established in calves exposed to infected sheep. When later challenged, these calves were fully susceptible to the virus.

Calves in direct contact with other calves given virus either naso-orally or parenterally failed to show signs of experimental infection. Subsequent challenge with stock virus showed that contact-exposed calves were fully susceptible.

Although viremia was demonstrated in sheep in two trials, exposure of calves to them at this time did not result in contact infection.

Tissue culture systems employing bovine and ovine thyroid tissues were developed. Preliminary studies indicate that Oregon (C24v) and Nebraska (M-833) agents replicate and produce cytopathic effects in monolayers of ovine thyroid cells. This cell system is applicable for virus neutralization tests. Studies are in progress to determine the susceptibility of these cell systems to virus isolates that fail to elicit cytopathic effects in other cell lines.

Procurement of over 40 calves by Caesarean section furthered development of the Specific Pathogen-Free (SPF) cattle herd. The opportunity was taken to study the possible presence of bacteria and viruses in the in utero environment. Bacteriological examination of the fetus, umbilical cord blood, and extra-embryonic fluids showed that these calves were apparently free of bacteria at time of section. Study of developmental changes in blood cellular elements and serum proteins were made at birth and early in post-natal life. Methods for rearing of these colostrum-deprived calves were refined.

Sterile swabs were used to obtain specimens from the amino-allantoic fluids, fetal, skin, anus, and nose. Samples of placental blood have been obtained from the umbilical cord. All samples were subjected to anaerobic and aerobic culture conditions. Blood agar and thioglycollate media were inoculated and incubated at 37 C for 48 hours.



Bacteria were present in cultures from four of 29 calves. Two isolates of non-pathogenic organisms were from the nasal area. In both calves the specimens were not obtained until after respirations had been initiated and the isolates were considered to be air borne contaminants. A gram positive cocci from the amniotic fluids and a coliform organism were isolated from two other calves. Possible contamination of a calf was known to result from an accidental perforation of the maternal intestinal tract. The efficiency of the bacteriological procedures was demonstrated by the isolation of bacteria from the nose, skin, and anal region of this calf.

The bovine placenta effectively interferes with the transfer of maternal antibodies to the fetus. Passive immunity is provided for the newborn calf by enteric absorption of colostrum antibodies. As the Caesarean derived calves were deprived of colostrum, this provided an opportunity to evaluate the functional development of the reticulo-endothelial system of the calf. Serum or plasma samples were collected from the blood samples obtained for hematology. Electrophoretic analysis of the serum (plasma) proteins are being utilized to give a qualitative evaluation of the various components, which are then quantitated by nitrogen determinations. This work is in progress.

In 1961, at the Iowa State University, Ames, Iowa, under a cooperative agreement with the USDA, it was found that, with the exception of the combination of the Sanders and PPLO agents and the delayed combination of Sanders and Nebraska MD agents, the series of experiments involving a combination of agents of the mucosal disease viral diarrhea complex produced a response which closely approaches the field syndrome than if these agents are inoculated by themselves. On the basis of these preliminary experiments one can hypothesize that mucosal disease as it is seen in the field probably is the result of an infection by a combination of specific agents plus possibly other factors. The proof or disproof of this hypothesis awaits further trials.

A definite, repeatable syndrome can be reproduced in calves by the inoculation of the Sanders agent. Furthermore, it has been observed that this agent can be serially passed from one calf to another with the typical syndrome appearing at each passage. Swine and goats showed no conclusive response to the Sanders agent. Sheep appear to be susceptible to the Sanders agent and develop a syndrome milder but similar to that seen in calves. Present information indicates that mice, guinea pigs and chicken embryos show no evidence of susceptibility to the Sanders agent.

In 1962 workers at the Iowa State University reported a viral agent was isolated from a calf from the Colglazier herd showing typical signs of mucosal disease. This agent was recovered from deep scrapings of involved Peyer's patches. Isolation was accomplished by four passages through primary cell cultures of bovine kidney and testicle cells. The virus produces distinct cytopathic effects in primary cell cultures of bovine origin. A plaque method was devised enabling a highly accurate titration method. Using this method,

cross neutralization tests against known strains of virus diarrhea agents were done. Results of these tests indicated the absence of any serological relationship between the newly isolated agent and two reference VDV virus antisera produced in rabbits. The virus is not pathogenic for mice by any route. There is indication that a disease syndrome is produced in cattle, although insufficient trials will not permit us to make definite claims.

Preliminary trials using fluorescent antibody techniques to detect specific viral antigen in cell cultures and frozen sections have been done. It appears that specific antigen can be localized in cell cultures. We are presently trying to improve our methods with the intention of applying this method to diagnosis and basic research including the study of non-cytopathic strains and their serological relationships.

Attempts to isolate enteroviruses from 128 animals located in eight different herds and ranging in age from 3 days to approximately 5 years have been negative. Serum samples collected from 37 animals either affected with mucosal disease or located in infected herds have failed to neutralize the Nebraska mucosal disease agent. Serum samples from experimentally infected calves or hyperimmunized rabbits have shown that only the Nebraska and the North Dakota agent antiserum are capable of neutralizing the Nebraska mucosal disease agent. Antiserum against the Sanders, Merrell, Indiana virus Diarrhea, C-80-K and C-24-V agents fails to neutralize the Nebraska mucosal disease agent.

Many combinations of agents isolated from the mucosal disease-virus diarrhea complex have been used in an attempt to reproduce a typical case of mucosal disease terminating in death. However, our experimental trials have not been successful in this regard.

In 1961, the University of California, at Davis, California, in cooperation with the USDA, reported a filterable agent, which appears to be viral in nature, has been isolated from two different cows out of some 30 cows clinically diagnosed as suffering from bovine lymphosarcoma.

Cattle experimentally infected with infectious bovine rhinotracheitis (IBR) virus showed detectable antibodies after 44 months. Studies to date have been encouraging enough that a CF serology test may be devised to detect IBR antibodies.

A preliminary survey by means of serum neutralization in tissue culture has been initiated in the comparison of some cattle diseases of the United States to those which exist in Germany. Attempt to isolate the virus diarrhea virus of cattle in tissue culture from materials obtained from clinically diagnosed cases of virus diarrhea was not successful. Studies with the "viral" agent of bovine abortion were made in regard to its behavior in young calves and possible placental transmission.



In 1961 the Colorado State University, at Fort Collins, under a cooperative agreement with the USDA, reported that twenty cattle were continued on an experiment in a large animal virus isolation laboratory at Fort Collins to determine the rate of decline of antibody titer following inoculation with virulent virus of infectious bovine rhinotracheitis. During the year 3 animals showed 1000 fold reductions of antibody titers after 7 to 10 months. Their immunity was challenged by intratracheal inoculation of virulent virus. One developed typical symptoms of the disease, 2 showed increase in body temperature only, and 3 showed anamnestic response by augmentation of titers. The remaining 17, with the exception of the 4 controls, still had high titers 18 months after intratracheal, intramuscular, or intravenous inoculations. Seemingly there is no significant difference in titers as a result of different route of inoculation.

By the use of the fluorescent antigen-antibody technique, a study of virus infected bovine kidney cells in a tissue culture system showed no evidence of virus aggregation forming the inclusion body. Materials were received from 48 outbreaks for virus isolations from 11 States and from 280 cases for serum neutralization tests.

In 1962 studies were continued at the Colorado State University under the cooperative agreement, and during the year the serum neutralizing titers of cattle which are kept in the isolation units, did not show significant reduction. There was no evidence of difference in neutralizing antibody level between the two groups of animals infected intratracheally and those infected intramuscularly.

In a study of abortion of cows in relation to IBR infection, the isolation of IBR virus from aborted fetuses was the first stage of the work. Field reports of abortions were investigated. Fetuses were collected, and different organs of the fetus were used for isolation of IBR virus. The virus was isolated from the lung tissue of the fetus only. If more fetuses could be obtained it might be possible to isolate the virus from other organs.

In studying IBR epizootics in ranch cattle, fifty animals (47 deer from 1 to 6 years of age, and 3 elk from 1 to 2 years of age) were gathered together for testing. Blood samples from these animals were collected. These animals will be challenged or infected with IBR virus to determine their susceptibility and what sign(s) of sickness, if any, are manifested. Since deer, elk, and cattle cohabitate in pasture and range areas, the susceptibility of deer and elk to IBR may be an important factor in determining transmission of the disease.

In 1962, at the National Animal Disease Laboratory at Ames, Iowa, naturally occurring cases of BVD or MD have been studied in the NADL herd from which one agent was recovered and in one farm herd from which 4 probably identical agents were recovered. Inoculation of the NADL agent into an experimental animal was studied for development of clinical signs, blood changes, virus recovery, and production of neutralizing antibody. Periodic bleedings have

been made from the NADL herd as well as from the farm herd. Isolation of additional agents from the respective herds was attempted by making rectal swabs and leucocyte cultures from apparently normal cattle. This resulted in the isolation of an agent from 1 out of 5 rectal swabs taken in the farm herd and from 12 out of 19 rectal swabs representing all cattle in one NADL barn. In addition, 5 isolates were obtained from leucocyte cultures out of the 19 apparently normal cattle housed in the NADL barn.

Virus neutralization studies indicate that the agents isolated from rectal swabs are antigenically different from those isolated by leucocyte cultures. They also appear distinct on the basis of rapidity and type of CPE produced in tissue cultures.

Five viral agents identified with bovine virus diarrhea (BVD) or mucosal disease (MD) have been obtained from other laboratories for storage in the repository and subsequent use as reference material. The Oregon virus diarrhea agent (C24V) has been selected as the prototype since it is the one most commonly used in other laboratories.

A bovine kidney cell line designated National Laboratory Bovine Kidney (NLBK) was developed as a laboratory tool. It appears to be very useful because it is susceptible to the prototype virus as well as several other viruses of bovine origin.

#### F. Mastitis

In 1961 research was continued at the Animal Disease Station at Beltsville, Maryland, on the development of a medium for the titration of lactenin. Beef infusion, when added to a medium containing casin hydrolysate, vitamins, glucose, and various salts, permitted the growth of Streptococcus pyogenes. The growth factor will pass through a cellophane membrane, will migrate to the cathode chamber in an electrodialysis cell and is stable to autoclaving or concentration at 80 C. in a flash evaporator. The growth factor is not associated with carbohydrates or minerals present in the ash. Several amino acids, purines and a pyrimidine have been tentatively identified by chromatographic methods but have not been related, as yet, to the growth factor.

In 1962, at the National Animal Disease Laboratory, Ames, Iowa, research on mastitis was continued on the study of factors in milk which inhibit the growth of Streptococcus agalactiae. The inhibitory activity of milk was not affected by preparing whey by treatment with acid or rennin and centrifuging the wheys at speeds up to 17,000 rpm (34,800 X G). No loss in inhibitory activity was caused by dialyzing whey against de-ionized water or a 0.02 M phosphate buffer of pH 7.0 for 48 hours at 5°C. However, electro-dialyzing whey at room temperature for 7 hours at constant current (100 m.a.) resulted in a decrease in activity of at least 50 percent.



When milk from infected quarters was titrated raw, the dose-response curve typical for the inhibitory activity of raw milk from non-infected quarters was altered because of increased acid production. This increase in acid production was eliminated by pasteurizing the milk from the infected quarters before titration.

Research was also continued on the development of a culture medium of defined composition to grow streptococci of serological Groups A and B for use in assaying the growth inhibitory factors in milk. A culture medium, composed entirely of known constituents except for 1 mg per milliliter of crude egg albumin will support good growth of Streptococcus pyogenes. Washed cell suspensions which were grown in Difco brain-heart-infusion broth were used as a source of inoculum. Purified egg white fractions such as conalbumin, ovalbumin and perhaps lysozyme will substitute for the crude egg albumin. This is the same basic medium used for microbiological assay of amino acids and is described in Cornell Experiment Station Bulletin No. 337, 1955.

The same medium without the egg albumin, supported good growth of several cultures of Streptococcus agalactiae. These organisms are less fastidious than the group A cultures and will grow in a medium in which all components are known.

Growth of these two groups of organisms can be measured with speed and accuracy by titration of the acid formed. Both groups converted 90 or more per cent of the glucose fermented to lactic acid.

Four strains of Str. agalactiae were shown by analysis of their fermentation products to be homofermentative lactic acid bacteria.

In 1961, the University of California, at Davis, in cooperation with the USDA, in studies in a limited number of animals, the following results may be of significant value for future studies on the big and important research problem of mastitis.

A small number of Aerobacter aerogenes may induce clinical mastitis in a normal mammary gland. Repeated small doses did not lead to an intensification of the clinical response but may have contributed to development of an infection of 20 days duration.

A pre-existing chronic inflammation caused by Ps. aeruginosa gave some protection against development of an acute clinical phase of mastitis following the introduction of 4,000,000 A. aerogenes. A persistent infection was not established for the longest period of residence in any gland was 9 days.

In an attempt to counteract A. aerogenes infection, the inoculation of extremely large numbers of micrococci led to a high and somewhat persistent level of leukocytosis accompanied by swelling and clots. This was followed in 5 days by disappearance of the A. aerogenes infection, and on the 6th day the micrococci disappeared.

The introduction of approximately 1 million dead A. aerogenes organisms into normal lactating mammary quarters induced a leukocytosis of 4 days duration with the peak number of cells in foremilk reaching 4.0 to 5.0 million/ml. Viable organism in the same dose level stimulated cellular responses in 8½ hours of 60.0 million cells/ml and in addition swelling of the gland was observed for 3½ days and rectal temperature reached 106.0°F at 8½ but quickly returned to normal. During the period of persistence of the A. aerogenes within the gland a see-saw pattern of cellular activity was seen with two or more peaks of considerable magnitude followed immediately by a rapid fall in cell numbers. Presence of A. aerogenes in the milk was demonstrated mostly only by incubation of the milk before culturing on the surface of blood agar plates.

The information gained from other limited clinical, bacterial, and chemical studies on mastitis points to the value of gathering data during the early phases of the response to bacterial infection in the mammary gland in order to better understand the natural mechanisms for defense of the total animal. The response of the animal to infection is immediate and, therefore, future studies should include examinations at frequent intervals during the first few hours.

In 1962, the University of California, cooperatively with the USDA, studied a culture of Aerobacter aerogenes originally isolated from the bovine udder in 1959 which was employed in the production of experimental mastitis. It was demonstrated that less than 100 organisms, when introduced into the teat and gland cistern of an absolutely normal lactating mammary gland, are capable of inducing in 10-15 hours an acute mastitis with an associated systemic reaction. It was also clearly demonstrated that a pre-existing leukocytosis, even of low order, has significant protective benefits. Systemic signs, such as elevated body temperature and anorexia, and local signs, such as detectable swelling of the inoculated gland, are prevented from developing when leukocytes are present in the udder secretion at the time of introduction of the culture. The inoculum, however, causes an increase in exudation of neutrophils so that for several milkings the cell count/ml. is increased significantly, although visible signs such as clots in the milk may not occur.

The experiments reported here strongly indicate that mammary glands must be completely free of even a minimal inflammatory reaction before coliform-type organisms are able to produce acute mastitis. Patency of teat canal is also involved. The most apt to have patent streak canals are the older animals and since the older animals most regularly have a leukocytosis from repeated udder stress, such cows at the same time are protected against coliform mastitis. Application of extensive intramammary therapy to such cows may produce complete freedom of such glands from leukocytosis and render the animals susceptible to coliform mastitis.



### G. Shipping Fever

In 1961, at the Animal Disease Station, Beltsville, Maryland, as a continuation of studies on the etiology and transmission of shipping fever, 3 Holstein calves were injected intramuscularly with live para-influenza 3 virus, three with killed Pasteurella multocida and P. haemolytica, and three with a combination of the live virus and killed Pasteurella spp. Para-influenza was not transmitted from the injected calves to contact controls as determined by hemagglutination-inhibition test and attempts at virus isolation.

The 9 injected and 5 control Holstein calves were assembled in a feed lot with 27 Angus calves, of which several were showing clinical signs of shipping fever. P. multocida, P. haemolytica, and para-influenza 3 virus were transmitted to the Holstein calves by contact. P. multocida, P. haemolytica and para-influenza 3 virus were isolated from 8, 13, and 9 Holstein calves respectively. The injection of the above three agents before contact exposure could not be adequately evaluated under the conditions of the experiment.

In 1962, at the National Animal Disease Station, Ames, Iowa, fundamental studies on the nutrition and metabolism of Pasteurella sp., believed to have etiological relationship to shipping fever, are in progress. Blood agar base containing hemin was the best medium tested for the enumeration of P. multocida and P. hemolytica, producing the highest, least variable counts, while gelatin-saline was the best diluent for counting procedures.

Studies designed to investigate experimental infection produced by para-influenza-3 (SF-4) virus in laboratory animals and in various tissue culture systems are in progress. Parainfluenza-3 virus multiplies readily in the amniotic sac of 13- to 14-day-old chick embryos, before and after serial passage, without losing its characteristic pathogenicity for primary cultures of embryonic bovine-kidney cells. Infection was not maintained by serial passage of egg or tissue culture virus in the allantoic cavity of younger embryos. Attempts to demonstrate plaque formation by parainfluenza-3 virus in primary monolayer cultures of embryonic bovine-kidney cells with agar overlays were not successful.

### H. Leptospirosis.

In 1961, at the Animal Disease Station at Beltsville, Leptospira pomona was grown in a medium in which the whole serum had been replaced by 1% albumin. Vitamin B<sub>12</sub> was required for growth. Ammonia may be a major source of nitrogen.<sup>12</sup> No change in antigenic characteristics occurred.

Anaplasmosis infection did not protect cattle from infection with Leptospira pomona. Stress from leptospirosis triggered recurrences of anaplasmosis.

Leptospira pomona infection was fatal to chinchillas in as early as 6 days. Two animals inoculated with bovine kidney tissue from which Leptospira pomona was isolated by culture failed to become infected although they were susceptible to infection upon subsequent challenge.

No new infections have occurred in the Jeanerette Dairy herd where a long term vaccination study is in progress.

In 1962, at the National Animal Disease Laboratory, Ames, Iowa, Leptospira pomona and 11 other major serotypes have been successfully cultured through an indefinite number of transfers in a medium whose major and most critical components are bovine albumin (Dubos oleic albumin complex DIFCO, Detroit, Michigan), Vitamin B-12, and ammonium chloride.

Maximum growth is achieved at a concentration of 1 percent albumin, although continuous subculture is possible at 0.25 percent albumin. Vitamin B-12 is the only vitamin which has been shown to be an absolute vitamin requirement in this specific medium. This need can best be demonstrated after subculture in the absence of added culture medium B-12. The amount of B-12 bound to the albumin has not been determined. No requirement for thiamin in this medium could be demonstrated. Consequently, thiamin has been deleted from the growth medium.

The ammonium ion source of choice is ammonium chloride. It is stimulatory at low levels, nontoxic at high levels, and cannot be replaced with monovalent cations. The deletion of ammonium ion still allows for continued growth in the presence of albumin.

Magnesium ion was found stimulatory to growth, but is not an absolute requirement for growth in the presence of the protein albumin. The stimulatory activity is not replaceable with divalent cations. The optimal level of sodium chloride in this medium is in the range of 0.27 - 0.37 percent.

The absolute need for trace metals is still in question and may remain so until deletion of the protein is achieved. The same is true of l-cystine.

Minute inocula of the order of .25 percent as opposed to conventional 10 percent inocula will attain maximal cell crops during extended incubation periods in the current medium.

The growth supporting activity of the medium is not diminished by temperatures of 56° to 70° C. Complete destruction of growth supporting activity is seen at 82° to 84° C. after 30 minutes exposure.

This medium has been prepared in semi-solid form containing 0.2 percent regular agar and permits maintenance of stock cultures for prolonged periods of time.

The vaccination study in the Jeanerette dairy herd has continued. The fall 1961 bleeding was accomplished as planned but extensive personnel changes at Jeanerette interfered with the spring bleeding. To date no evidence of infection has been observed in either the vaccinated or nonvaccinated animals. The herd is gradually becoming seronegative through the coincidental removal of reactors in the routine culling process.



# I. Epizootic Bovine Abortion.

In 1962, the University of California, Davis, under a cooperative agreement with the USDA, conducted investigations of epizootic bovine abortion (EBA). The studies under way include 1) attempts to ascertain whether the virus of EBA can induce abortion when administered either orally or nasally. These are believed to be the natural routes of infection. Should it be shown that the virus is capable of producing abortion when exposed to cattle through either or both of these avenues, it will remove any doubts that it might not be the sole cause of the condition. 2) attempts to determine whether cattle recovered from infection with the EBA virus are refractory to abortion when challenged with virulent virus. Such information is fundamental to any prevention program based on immunization. 3) Field trial studies to establish whether multiple injections of an inactivated EBA virus vaccine preparation will confer immunity to abortion. Contemplating that a viable agent will be needed to produce a satisfactory immunity, efforts are currently under way to attenuate the EBA virus by serial passage in tissue culture for possible use as an immunizing agent. 4) Epidemiological studies to ascertain whether the EBA virus is tick-transmitted and whether ticks are the reservoir of the virus in nature.

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FOOT-AND-MOUTH DISEASE AND OTHER  
EXOTIC DISEASES OF CATTLE  
Animal Disease and Parasite Research Division, ARS

Problem. Responsibility for protection of the Nation's livestock industry against diseases, including those of foreign origin, was delegated to the USDA in 1884. Thereafter, contagious bovine pleuropneumonia eventually was eradicated from the United States, thus reopening European markets for practicable, scientifically justified barriers against introduction of such dangerous exotic diseases as foot-and-mouth disease and rinderpest. The Plum Island Animal Disease Laboratory was established for scientific support of measures for protection against these and other foreign diseases of animals, following the direct threats of spread of foot-and-mouth disease from Mexico and Canada (1946-1954). Foot-and-mouth disease, which is capable of reducing over-all productivity by 25 percent in areas where it becomes established, persists in most major livestock producing countries, except Central and North America, Australia, and New Zealand. Rinderpest continues to be a serious disease problem in Africa and Asia; it is capable of killing 90 percent or more of the cattle that are exposed to it. Other diseases, such as contagious bovine pleuropneumonia, Rift Valley fever, and East Coast fever, continue to exact severe tolls in other parts of the world. Possibilities of entry of these diseases into the United States continue, despite all precautions, primarily because of the progressively increasing scope, speed, and extent of modern international transportation. The purposes of the Plum Island Laboratory are development of basic information applicable to protection of the Nation's livestock from foreign animal diseases; development and maintenance of competence in diagnosis of these diseases; and fundamental research on the biological, chemical, and physical properties of the infectious agents that may be useful in prevention, control, and eradication of these diseases.

USDA PROGRAM

The Department has a continuing long-term program involving veterinarians, biochemists, biophysicists, microbiologists, and pathologists, engaged in basic and applied research in this problem area. All of this research is being conducted on the following diseases at the Plum Island Animal Disease Laboratory, Greenport, Long Island, New York, except for supplemental field studies on vaccines in the Netherlands.

The Federal scientific effort devoted to research in this area, conducted solely at the Plum Island Animal Disease Laboratory, totals 33.3 professional man-years. This effort is divided among sub-headings as follows:

Pathological investigations of foot-and-mouth disease in cattle 1.0

Fluorescent antibody techniques 1.0

Diagnostic investigations 3.0

Susceptibility of cell lines 0.5

Production and maintenance of standardized reference stock of virus and homologous antisera 2.3



Carrier state in convalescent animals 0.5  
Parasites in transmission of foot-and-mouth disease 0.5  
Foot-and-mouth disease vaccines 4.0  
Antigenic variations of foot-and-mouth disease viruses 1.0  
Production of foot-and-mouth disease antibody *in vitro* 0.5  
Immune response to various types and subtypes of foot-and-mouth disease virus 1.5  
Quantity production of foot-and-mouth disease virus 2.0  
Microcinematography of infected cells 0.5  
Pure stable lines of culture cells 0.5  
Purification of foot-and-mouth disease virus 2.0  
Chemical and physical characterization of foot-and-mouth disease virus 1.0  
Interaction between foot-and-mouth disease virus and host cells 1.0  
Genetic biochemistry of foot-and-mouth disease virus 1.0  
Effects of chemical and physical environments of foot-and-mouth disease virus 1.0  
Preservation of foot-and-mouth disease virus 1.0  
Rinderpest 2.5  
Transmission of foot-and-mouth disease virus in semen 1.5  
Survival of foot-and-mouth disease in meat and meat products 2.0  
Susceptibility of wild species to foot-and-mouth disease 0.5  
Adaptation of foot-and-mouth disease virus to poultry and embryonating chicken eggs 1.0

Public Law 480 funds equivalent to \$11,572.21 have been made available to the Turkish Ministry of Agriculture for a 2-year study of tissue culture of indigenous strains of foot-and-mouth disease virus and experimental field vaccination.

\$78,594 have been allotted to the Biological Institute, Sao Paulo, Brazil, for a 5-year study of tissue culture of indigenous strains of foot-and-mouth disease virus and experimental field vaccination.

#### RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

Experimentation with the virus of foot-and-mouth disease in the United States essentially is prohibited by law, except at the Plum Island Animal Disease Laboratory. Experimentations with the causative agents of other communicable, foreign, or exotic diseases of cattle in the United States is similarly prohibited generally by federal regulations. Consequently, the State Experiment Stations are not working with diseases in this category.

Insofar as is known, foot-and-mouth disease is the only one of the foreign diseases of cattle in which American industry has manifested notable interest. Although experimentation with foot-and-mouth disease is prohibited in the United States, except at Plum Island, at least two U. S. biological firms are known to have initiated vaccine production programs in South America, with plans for limited corollary research. It is estimated that no more than about 5 professional man-years are so engaged.

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

##### A. Pathological investigations of foot-and-mouth disease in cattle.

In 1961, studies on the frequency of atypical (non-vesicular) lesions of foot-and-mouth disease in cattle were temporarily discontinued due to emphasis on other lines of cytological investigation. Spontaneous occurrence of so-called metastatic calcification syndrome among guinea pigs used for FMDV experimentation necessitated study of this disease on several occasions. Nephritis was found consistently in affected animals, but cytologic study and volumetric determinations of the parathyroid glands failed to show evidence of parathyroid hyperactivity secondary to renal damage. It was concluded that the calcification syndrome is of dietary, rather than renal origin, and that the secondary kidney lesions probably result from excretion of excess phosphates of dietary origin.

In 1962 work on this project was limited to routine service work in histopathological diagnosis performed in connection with work in other projects. In addition, a large collection of kodachrome slides (256) and prints illustrating animals affected with exotic diseases was selected and furnished to ADE and the Economic Research Service.

##### B. Fluorescent antibody techniques.

In 1961, two techniques were developed which may be used for demonstrating, localizing and identifying FMDV in tissues. In the indirect technique, the system consists of treating of FMD-infected tissue-culture cells with rabbit FMD antiserum, following which commercial fluorescein-conjugated sheep anti-rabbit globulin is added. The addition of commercial rhodamine bovine albumin to the FMD antisera and to the conjugate enhances the readability of positive reactions against normal rabbit serum controls by eliminating yellowish non-specific fluorescence. The direct method involves fluorescein-conjugated globulin from guinea pigs and bovine FMD antiserum (treated with liver powder and bovine albumin, to which rhodamine has been added to reduce non-specific fluorescence), with FMD-infected cell cultures. With these two techniques, the results indicate that high-passage tissue-culture virus may develop a broad antigenic spectrum. The results to date suggest these procedures may be useful in selection of strains of virus for vaccine production.



In 1962, the mass of antigen-antibody combination was found to be the most essential factor in obtaining fluorescent antibody reactions with foot-and-mouth disease virus (FMDV) that could be evaluated microscopically. A system including probable viral and cellular antigens combining with appropriate antibodies in convalescent bovine serum was developed for practical detection of cattle recovered from foot-and-mouth disease (FMD). The test developed is specific for FMD, but does not discriminate between virus types.

An indirect fluorescent antibody technique using rabbit-rinderpest serum proved satisfactory for demonstration of rinderpest antigen(s) in infected bovine kidney tissue cultures.

#### C. Diagnostic investigations.

In 1961 preparedness for laboratory assistance in diagnosis of foot-and-mouth disease, and differentiation from vesicular stomatitis and vesicular exanthema, two clinically similar but distinct diseases, were considered of basic importance in the Plum Island program. Work is constantly under way in fundamental explorations aimed toward development of practical, accurate, and rapid means of identification of virus and specific antibodies.

In 1962 there was no report for work on this project.

#### D. Foot-and-mouth disease vaccines.

In 1961 the optimum conditions with regard to media, quantity of inoculum and time of harvest of virus grown in tissue cultures for large scale production of vaccine have been determined. Virus grown in bovine kidney tissue culture in bottles has produced a satisfactory vaccine for cattle at comparatively low cost and with potentially superior qualities. To date, virus grown in suspensions of trypsinized kidney cells has not been satisfactory for use in vaccine, apparently due to the low virus titers obtained by this method of propagation.

Continuation of studies on the safest methods of inactivation by formaldehyde has resulted in more rigid test procedures than previously were considered necessary. The testing of vaccine in 10 liter lots revealed new conditions which were not obvious when working with smaller experimental lots. In conjunction with these studies a method of testing for residual virus using tissue-culture methods is being explored, with indications that such a method may afford greater assurance of safety than testing in cattle only.

Correlation of measures of potency of vaccine in guinea pigs and cattle is continuing with indications that the response in vaccinated guinea pigs will permit estimation of the probable response in cattle to the same vaccine. A study of this type requires several simultaneous comparative trials in order to determine degree of correlation.

A mineral oil complex is being compared with standard aluminum hydroxide gel as an adjuvant in the vaccine. To date, data indicate the new adjuvant is as good as and possibly superior to the aluminum gel. When the mineral oil complex was used, the antibody response was 100-fold higher in some animals.

Vaccines produced for use in cattle are being tested concurrently in swine. There appears to be a fundamental difference between the two species in vaccine response; swine generally have responded poorly to vaccine produced from virus propagated in tissue cultures. There are some indications, however, that the response in swine may be a function of the adjuvant used in the vaccine and the route of injection of the product.

Comprehensive studies under way in Europe on the duration and extent of immunity in cattle following vaccination with FMD vaccine require long periods of time and many animals. Such investigations are difficult if not infeasible under laboratory conditions. An ADP representative is stationed in Amsterdam, as Chief of the Division's European Mission for Research on Animal Diseases, and such studies are in progress in the Netherlands in cooperation with Dutch researchers. Tests involving 2 herds of Friesian cattle have been in progress for approximately 2 years. Serologic studies in the 2 herds have been continued. Results obtained during the past year have warranted extension of the program to include additional herds. This will afford more significant numbers of animals for challenge with virulent FMD virus and permit correlation of resistance to infection with the index of immunity as measured by virus-neutralization tests of serums.

To obtain sufficient animals for challenge, 10 additional herds have been included in the study. The total number of animals under observation is approximately 400. It is estimated that of this number about 40 annually will become available for challenge. The substantial increase in the number of samples to be tested as a result of this change has justified comparative studies between the virus-neutralization test, depending upon complement fixation, and another test, depending upon changes in pH.

Serums of animals which have experienced two or more annual vaccinations continue to show a high level of antibodies. Two animals showing high levels of antibodies were resistant to challenge with virulent virus, as indicated by absence of generalized infection. Results to date promise eventual development of sound techniques for evaluation of vaccines prepared by various methods, and scientific application of vaccines in the field.

In 1962 cattle vaccinated with inactivated FMD vaccine containing emulsified oil as an adjuvant developed a significantly better protector than cattle receiving a similar product containing aluminum-hydroxide gel as an adjuvant. Study of virus-neutralizing antibodies revealed high antibody levels at 9 months in cattle vaccinated with the emulsified oil preparation, whereas, cattle vaccinated with vaccine containing aluminum-hydroxide gel had low antibody levels at four months. Challenge of these cattle by exposure to live virus showed that the cattle which received the oil emulsion vaccine were better protected.



#### E. Antigenic variations of foot-and-mouth disease viruses.

In 1961 the susceptibility of mother mice to FMDV began to decline about 3 weeks post-partum until the 5th week when they became almost completely resistant. Substantial reduction of the number of suckling mice 2 days before inoculation of mother mice appears to increase their resistance. Cells from peritoneal exudate of susceptible mother mice exposed in vitro to FMDV have shown no diminution of phagocytic activity (for yeast) nor was virus absorbed or propagated in these cells. Increasing the number of white cells in the peritonea of mother mice before infection with FMDV did not appear to alter their susceptibility.

In 1962 factors were investigated that might affect the susceptibility of mother mice to FMDV. Virus from tongue epithelium of infected steers and virus from the first few passages in tissue culture produced a low mortality in mother mice, but after 6-8 serial passages in tissue culture the virus killed 40-70% of the mothers. Beginning approximately three weeks post partum, mother mice, which were resistant to the virus before becoming pregnant, gradually became resistant again. The ability of mother mice to respond immunologically is impaired as demonstrated by a hypersensitivity response to bovine serum. The response is lower in both mother mice sensitized seven days post partum and in mother mice sensitized before being bred.

Basic studies under this project have shown that bovine serum antibody may be assayed in the complement-fixation test using guinea pig vesicular fluid as an antigen. Study of sheep serum has shown it to have good complement-fixing antibody and apparently none of the qualities of bovine serum that make CF tests difficult. Comparison of antigens from different sources such as tissue culture and guinea pig vesicular fluid have led to studies to establish antigen standards. Such standards are essential for the serological testing of vaccine antigenicity.

#### F. Immune response to various types and subtypes of foot-and-mouth disease virus.

In 1961 serological studies were continued on a group of cattle three years after infection and a detectable level of circulating antibody has persisted. Studies have included examination of the animals for latent virus infection to determine whether the persisting antibody level may be due to intracellular virus which has continued to stimulate antibody production. Another approach has been inoculation of pituitary hormones to produce physiological stress which might cause animals to shed latent virus. Although the results to date have been negative, the existence of residual virus in these animals has not been disproved.

In 1962, detailed studies of transfer of immunity from vaccinated cows to their calves have shown this to be through the colostrum only. It has been shown that a calf is born free of serum gamma globulin and only receives it through maternal colostrum. The duration of this passive immunity is dependent on the antibody content of the colostrum received by the calf when it first nurses.

#### G. Quantity production of foot-and-mouth disease virus.

In 1961 work was initiated on devising methods for rapid and economical production of large quantities of FMDV in surviving kidney-cell suspensions. Steps in the operation being studied included (1) areas of renal tissue providing best viral growth, (2) methods for mincing large quantities of tissue, (3) trypsinizing procedures, (4) types of culture vessels, (5) media, (6) growth conditions, including cell concentration, depth of culture, agitation and alteration, and (7) fragmentation of infected cells for virus release.

In preliminary work, cultures of FMDV, type A, strain 119, have been attained with infectivity titers somewhat higher than the titer considered satisfactory for vaccine production in foreign countries ( $10^6$  infectious units per ml.). Methods of production of large quantities of FMDV, type A, strain 119, in bovine kidney cells cultured on glass have been improved by providing better conditions for the cells for viral growth. This has resulted in a 50 percent increase in susceptibility to infection and in virus yield.

In 1962 methods were developed for rapid and economical production of large quantities of foot-and-mouth disease virus (FMDV) in stationary suspensions of trypsin-dispersed bovine kidney cells in a simple medium. Yields of between  $10^7$  and  $10^8$  plaque-forming units (PFU) per milliliter were obtained from serum-free cultures containing approximately a million and a half viable trypsin-dispersed cells per milliliter. Yields of up to  $10^{6.9}$  PFU were obtained from simple cultures of finely minced calf kidney tissue provided cell debris was removed from the minced particles by trypsinization.

#### H. Microcinematography of infected cells.

In 1961 tissue cultures infected with type A, strain 119, FMDV were photographed. The cytoplasm of infected cells contracts around the nucleus leaving small branching streamers of protoplasm attached to the glass resulting in a bush-like or tree-like alteration of the cytoplasm. The term "arborization of the cytoplasm" was applied to this stage of the degenerative change. Normal cells undergoing mitosis also contract but their cytoplasm fails to undergo arborization. After the degenerating cells have undergone arborization, they undergo a form of activity described as "boiling", and then detach from the glass. Critical evaluation of preliminary films indicated the need for substantial modifications of the equipment to provide longer exposure time at higher magnification. This was accomplished, and considerable experimentation with different methods of film processing was made to provide high-quality film.

In 1962 improvements in microcinematographic technique have resulted in production of films satisfactory for demonstration and possible distribution. Microcinematographic studies have been made on growth of normal primary calf-kidney cultures and cultures infected with foot-and-mouth disease (FMDV) and rinderpest virus. Films prepared have been shown at scientific meetings. The phenomenon of cell survival was noticed and confirmed in the course of the above studies. A small portion of the cell population in primary calf-kidney cultures resists the action of FMDV. The cells not destroyed by the virus continue to multiply. Virus in low titer persists in the surviving cultures but a small-plaque moiety becomes predominant. Studies on changes developing in virus populations persisting in surviving cultures are being made.



# I. Pure stable lines of culture cells.

In 1962, a manuscript was prepared with abstract as follows: Continuous culture of lamb testis cells on glass and in agitated fluid suspension cultures has been achieved several times. No obvious cell alterations occurred during more than 40 serial passages. The culture system appeared to be well suited for production and assay of foot-and-mouth disease and some other animal viruses. Several additional established cell lines from outside sources were tested for susceptibility to foot-and-mouth disease virus with negative results.

# J. Purification of foot-and-mouth disease virus.

In 1961, a rapid method of centrifugation for purifying FMDV was developed which combined isodensity separation immediately below a moving zone separation. A preformed density gradient of cesium chloride enabled the virus to band into a narrow zone within 4 hours at 37,000 rpm in a swinging bucket tube. The viral light-scattering zone when removed at a concentration of 8-fold had  $47 \pm 16\%$  of the original infectivity and contained virus particles as revealed by electron microscopy. The cesium chloride isodensity value of FMDV of  $1.43 \pm 0.01$  g/ml was significantly higher than that of protein contaminants.

Combinations of basic types of centrifugation include sedimentation of FMDV through an interface formed by an aqueous phase and an immiscible organic fluid. Separation of virus from contaminants appears to depend upon hydrated particle densities and specific denaturation by organic fluids, in contrast to the dependence upon anhydrous particle densities in cesium chloride gradients.

Certain viruses, particularly bacteriophages, are stable only in the presence of magnesium ions. This ion, however, was found to have no stabilizing effect and possibly even detrimental to FMDV infectivity. This observation prompted use of the chelating agent, sodium ethylene diamine tetraacetate (EDTA) to remove bivalent cations from the highly purified virus described above. In 1% EDTA infectivity was essentially constant over a 55-day period at  $-60^{\circ}\text{C}$ ; longer periods have not been investigated. EDTA can be readily removed by dialysis.

In 1962, a new ultracentrifugation technique termed organic interface centrifugation was perfected. It combines in one run, moving boundary centrifugation, isodensity purification and organic extraction. Foot-and-mouth disease virus is being purified and concentrated from infectious tissue culture fluids by procedures incorporating alcohol precipitation, organic extractions, cesium chloride density gradient centrifugations and organic-interface centrifugations. The average weight of virus obtained from 10 liters of fluid was 100 micrograms based on electron microscope counts. This work will be continued until foot-and-mouth disease virus of at least 95% purity is obtained. Progress has been limited by the lack of a virus production unit at Plum Island.

# K. Chemical and physical characterization of foot-and-mouth disease virus.

In 1961, foot-and-mouth disease virus (FMDV), type A, from infected guinea-pig foot-pad vesicular fluid was examined for fine structure in both phosphotungstic acid and uranium-shadowed preparations on carbon coated grids. Phosphotungstic acid penetration was less than that reported with many other viruses,

hence shadowed specimens were also used extensively to study the structure. Regular polyhedral models of 12, 20, 32, and 42 subunits were constructed and analyzed for distinguishing features exhibited by both virus and model. Mixtures of FMDV and bacteriophage which have approximately the same diameters, revealed that FMDV had smaller and more numerous subunits. The icosahedral model with 42 subunits was favored over the modified dodecahedral model of 32 subunits for FMDV, although there was no unequivocal evidence that these represented the ultimate structure of FMDV. A carbon shadowing device was modified to facilitate studies of ultra-structure. The improvement consisted of a tungsten metal spring for advancing the pointed carbon rod toward the cavity in the blunt rod.

In 1962, foot-and-mouth disease virus was compared in the electron microscope with a bacteriophage and Turnip yellow mosaic virus (32 subunits). The number of subunits in foot-and-mouth disease virus exceeded that of the bacteriophage, but the exact number could not be determined. Complications in ultracentrifugation calculations introduced by the use of variously shaped cells were simplified by using higher mathematical functions in tabular form. Tabulation of log values required for sedimentation coefficients permits rapid calculation of the minimum s-rate for sedimentation for a known volume.

#### L. Interaction between foot-and-mouth disease virus and host cells.

In 1961, cultured bovine cells, partially depleted of endogenous metabolites and maintained on a medium with glucose as the sole organic material, were examined for acid production by known chromatographic procedures. The kinds of acids detected and their rates of production were the same for both normal and infected cells. Lactic acid constituted about 90% of the total acidity with acetic acid being the only other acid identified during the 12-hour post-starvation period studied. Concentration of acetic acid was highest during the initial recovery period of the starved cells. Cells infected with FMDV yielded comparable results although acetate production appeared to persist for longer periods of time. No acids of the tri-carboxylic acid cycle were detected in either infected or uninfected cells.

Cells partially depleted of endogenous nutrients utilized pyruvate at a rate 2.5 to 4 times greater than glucose. The pathways of pyruvate metabolism were not clearly defined. Pyruvate was not appreciably metabolized oxidatively since oxygen uptake could account for only 6-29% of the pyruvate consumed and malonate only slightly decreased its utilization. Substrate pyruvate did not form lactate and was used to a greater extent as glucose concentration decreased. Glucose utilization was independent of pyruvate concentration. Virus infection did not change the rate of pyruvate utilization in contrast to the increase found in glucose uptake. Lactalbumin hydrolyzate did not yield lactic acid of itself but did stimulate glucose uptake and corresponding lactate formation. Acetate was not oxidized nor was lactate produced. Cells uninfected with FMDV maintained the same pattern of glucose metabolism as did uninfected cells.



Thin sectioning for electron microscopy has been started with the examination of sedimented cells from normal and FMDV-infected bovine kidney cultures as well as with tissues infected with rinderpest virus. Training of a technician in routines of fixation, embedding, sectioning and examination of the thin sections has been started. Studies are planned and in progress for following the synthesis of FMDV and of rinderpest virus in cultures.

In 1962 further chemical investigations of organic acids synthesized by bovine kidney culture cells grown in media with and without serum showed lactic acid to be the major component, accounting for 86 to 90% of the total, while acetic and pyruvic acids accounted for 7 to 9% and 3 to 6% respectively. Foot-and-mouth disease virus-infected cultures, independent of medium, produced more of these acids than uninfected cultures. Studies with cultures partially depleted of endogenous nutrients and exposed to serum-free medium indicated that acetic and pyruvic acids may be derived from an endogenous substrate other than glucose. The contribution of lactic acid to total acidity increased with increasing glucose concentrations.

The above chemical and other metabolic studies of glucose metabolism in bovine kidney culture cells were confirmed by radioisotope tracer studies. Extracellular acids account for nearly all of the  $C^{14}$  with over 90% residing in lactic acid. Intracellular isotopic activity, 1 to 3% of the total, was distributed among the cold trichloroacetic acid soluble pool, hot trichloroacetic acid, lipid and protein fractions.

Purine and pyrimidine analogues were studied for possible inhibition of foot-and-mouth disease virus synthesis in bovine kidney culture cells. Eight analogues at 250 ug/ml did not alter cellular metabolism measured in terms of oxygen consumption and glucose utilization. When tested for inhibition of virus synthesis 8-azaguanine, dithouracil and 5 bromouracil sometimes decreased the amount of recoverable virus. The latter compound gave the most consistent decreases, and the inhibition was not prevented by adding natural pyrimidines, uracil and thymine. Interpretations are not clear-cut because controls on virus stability in vitro with the analogues showed that 8-azaguanine and dithouracil had no effect, while 5 bromouracil caused significant losses in activity.

#### M. Genetic biochemistry of foot-and-mouth disease virus.

In 1961 the presumption that FMDV splits off infectious ribonucleic acid (RNA) when heated has been substantiated. The tedious job of removing all ribonuclease (RNase) from virus preparations was accomplished. The resulting RNase-free virus yielded infectious RNA when heated at 61 and 85 C. Bentonite has been found to eliminate the last traces of RNase from phenol-derived RNA. Such RNA may be stored for at least 3 months at -60C with undiminished infectivity. Heretofore, phenol-derived RNA retained full infectivity only when stored in liquid nitrogen.

The precision of the plaque assay for FMDV-RNA was determined. Randomizing older data for 100 replicate platings of RNA in two ways, i.e., into groups of 5 and 10, gave respective ranges with one standard deviation of  $27.2 \pm 21\%$  and  $54.3 \pm 16\%$ . New data for 50 replicate platings gave a range of  $17.5 \pm 27\%$ . Thus, plaque counts ranging between 17 to 54 are known to a precision of 27% or better 65% of the time. The plating efficiency of FMDV-RNA is increased nearly 10-fold by inclusion of slightly soluble substances in the inoculum. The best substance found thus far is Attasorb.

Work is continuing on an apparent RNase inhibitor in calf-kidney cell cultures. The RNase-like activity of cell extracts inactivating FMDV-RNA is potentiated markedly by heating, pH changes and by the protein-protein dissociating agent, p-chloromercuribenzoate (pCMB). The optimal temperature range for thermal potentiation is 55 to 65 C with activity increasing as much as 1000-fold. If sodium dodecylsulfate (SDS) is present prior to heating, the RNase-like activity of cell extracts is potentiated to a lesser degree. This is in accord with the finding that virus heated in the presence of SDS yields infectious RNA, with the hot SDS inactivating environmental RNase. Transient acidification or alkalization of cell extracts increases their RNase-like activity by 10-fold or greater. pCMB also effectively increased the ability of cell extracts to destroy RNA infectivity. Dissociated inhibitor and RNase do not recombine readily.

In 1962 kinetic curves for the thermal inactivation of FMDV-RNA, freed with bentonite of the last traces of ribonuclease, were determined at 7 temperatures over a 3 hour period. Such RNA is not pure and contains much cellular RNA and DNA. The rates were first order with 1.5-hour survivals of 0.095, 0.85, 0.45, 0.25, 0.08 and 0.006 at 1°, 26°, 37°, 45°, 55° and 61°C, respectively. Survival was only 0.001 after 10 minutes heating at 85°C. The primary structure, secondary structure and reactivity of infectious FMDV-RNA obtained from pure FMDV is under investigation.

#### N. Effects of chemical and physical environments of foot-and-mouth disease virus.

In 1961 a rate study of the inactivation of the virus with AEI (acetylenimine), BPL (betapropiolactone), and ETO (ethylene oxide), was performed at 23 C. To 250 ml. volumes of A-119 virus in the 89th tissue-culture passage 0.05% AEI, 0.05% BPL, or 0.5% ETO was added. Samples were taken at 2-hour intervals in the critical period of inactivation. In innocuity tests in pairs of cattle when 2 ml. of each sample was given IDL, the results were as follows: AEI in a concentration of 0.05% inactivated the virus in 22 to 24 hours, BPL in a concentration of 0.05% inactivated the virus in 12 hours, and ETO in a concentration of 0.05% inactivated the virus in 16 hours. The cattle which showed no signs of FMD were challenged after 14 days with  $10^{6.2}$  bovine ID<sub>50</sub> by the intramuscular route. In the cattle that had been injected with virus inactivated by AEI in a 24-hour exposure, no primary or secondary lesions of FMDV were found in the 14-day observation period. In the cattle inoculated with virus in the presence of BPL for 12 hours, both tongue and foot lesions developed during the post-challenge observation period. Injection



of virus in the presence of ETO for 16 hours permitted development of tongue and foot lesions in the challenged cattle. It appears from these studies that AEI is superior to BPL and ETO as an inactivant. Ancillary tests such as virus-neutralizing capacity of the serum of chickens injected with 1 ml. of the same preparations as given to the steers, and the virus-neutralizing capacity of adult mouse serums after injections with the inactive viral preparations confirm the foregoing assumption. The following titers in PFU/ml have been obtained with A, O, C, SAT-1, SAT-2, SAT-3, and Asia-1, respectively, after 89, 22, 22, 8, 8, 8, and 10 tissue-culture passages: 8.0, 7.8, 7.6, 8.2, 7.7, 6.3, and 7.3, in that order. The titers in mice as LD<sub>50</sub>/ml approximate the tissue culture PFU/ml.

In 1962, studies on chemical inactivation of foot-and-mouth disease virus (FMDV), 0.05% acetyleneimine (AEI), at a temperature of 23°C for 24 hours, inactivated FMDV propagated in tissue cultures. The effects of beta-propiolactone (BPL) were also studied and were found to be less reliable than AEI. FMDV preparations inactivated with AEI retain the greater part of their antigenicity while BPL is more severe on viral antigenicity as tested in cattle, chickens, and mice. Cattle used in the infectivity studies were injected in the tongue and were later shown to be immune when challenged by the intramuscular route with concentrations of virus as low as 10,000 bovine ID<sub>50</sub>.

Twenty nine cationic and anionic surface active agents were tested for virucidal effect against Type O-M11 FMDV. Only one chemical, methyl ethyl isoquinilinium chloride, was capable of destroying 3-4 logs of viral infectivity in 2 hours at 28 C, whereas others only inactivated between 1-2 logs of virus under the same conditions.

#### O. Preservation of foot-and-mouth disease virus.

In 1961 tissue culture-propagated virus and virus in bovine tongue-tissue suspensions were used for storage in three replicate experiments. To lots of tissue cultured A-119 virus in the 88th passage 5% gelatin, 5% sucrose, or 0.2% cysteine were added, respectively. Antibiotics in concentrations of 1000 units of penicillin and 1 mg. of streptomycin were added per ml. Samples of each preparation were stored at 37, 23, 4 and -50 C in flame-sealed ampoules. Lots of 10% suspensions of tongue tissue from cattle infected with A-119 virus in the 9th and 10th bovine passages were supported with 2% gelatin, 5% sucrose, 50% normal bovine serum, L.C. fluid (tissue culture medium), or tryptose phosphate broth. Antibiotics as described above, were also added. Samples of the preparations in flame-sealed ampoules were stored at 37, 23, 4 and -50 C. Infectivity studies of the various preparations were conducted in suckling mice. At the end of a year, it was evident that virus of tissue-culture origin with or without additives, was more stable at 37, 23 and 4 C than was virus in tissue suspension. Both forms of the virus retained the approximate original titers when held at -50 C for a year. Tissue-culture virus survived at least 7 days at 37 C, for 8 weeks at 23 C, and 12 months at 4 C. Virus in tissue suspension survived for 2 days at 37 C, 2 weeks at 23 C and 6 months at 4 C. The additives gave little if any supporting effect except in tissue suspension supported by 50% bovine serum at 4 C.

Cysteine had an apparently harmful effect. The freeze-drying was performed on an Edward's freeze-drying machine. It was established previously that a condenser temperature of  $-50^{\circ}\text{C}$  must be maintained in the primary phase of drying with a vacuum of 150 mm or less of mercury throughout a period of 20 hours, followed by a vacuum of 80 mm for the secondary phase of drying over  $\text{P}_2\text{O}_5$  for 4 hour period at  $23^{\circ}\text{C}$ . Virus from tissue cultures and in tissue suspensions was dried and stored in 10 ml ampoules in 4 ml. amounts. Additives in the following concentrations were used singly or in combination with tissue culture virus: 5% sucrose, 2% gelatin, 2% dextrin, 2% sucrose, 5% skim milk powder, 1% glutamate, and 5% normal bovine serum. Tryptose phosphate, L. C. fluid, 5% normal bovine serum, 5% glutamate, and 5% skim milk powder were used singly or in combination with virus in tissue suspension. All vials were flame-sealed under vacuum after drying. Titration of samples for infectivity was conducted in suckling mice. In the presence of perfect mechanical conditions it has been possible to dry the virus with only 0.5 to 1 log loss in infectivity. Tissue-culture virus without supportive preparations dropped in titer from 0.5 to 1 log of virus following storage for one year. However, for best storage 5% skim milk powder or a combination of 5% sucrose, 5% bovine serum, and 1% glutamate concentration should be added. Virus in tissue suspensions stored well for a period of a year with tryptose phosphate or L.C. fluid as diluents. Data is not complete for all lots of freeze-dried virus in storage for a year.

In 1962, type A-119 foot-and-mouth disease virus (FMDV) was propagated in tissue cultures and in the dermis of cattle tongues. Various proteins, carbohydrates, or salts of amino acids were added to both types of viral preparations. Samples of these materials in 4-ml. amounts in flame-sealed ampoules were held at  $37^{\circ}\text{C}$ ,  $4^{\circ}\text{C}$ ,  $23^{\circ}\text{C}$ , and  $-50^{\circ}\text{C}$  for periods up to a year. Virus produced in tissue cultures survived at least 7 days at  $37^{\circ}\text{C}$ , for 8 weeks at  $23^{\circ}\text{C}$ , and 12 months at  $4^{\circ}\text{C}$ . Virus in tissue suspensions survived 2 days at  $37^{\circ}\text{C}$ , for 2 weeks at  $23^{\circ}\text{C}$ , and for 8 months at  $4^{\circ}\text{C}$ . None of the additives significantly increased the storage survival. Virus from both sources, with or without additives, was stable for a year at  $-50^{\circ}\text{C}$ . All infectivity tests were conducted in mice.

Similar preparations of Type A-119 FMDV were prepared with various additives such as proteins, carbohydrates, or salts of amino acids. These preparations were freeze-dried in 4-ml. amounts in ampoules in the Edward's freeze-dryer and stored for a year at  $4^{\circ}\text{C}$ . In most instances the over-all loss of virus in processing and storage did not exceed a log in infectivity. There was no apparent difference in storage stability between the two sources of virus and the additives apparently did not appear to influence the storage qualities.

#### P. Rinderpest.

In 1961 some dogs inoculated with rinderpest virus developed antibodies to rinderpest and resisted challenge with distemper virus. Cattle inoculated with measles and distemper viruses failed to develop antibodies against rinderpest virus. Inoculation of monkeys with distemper and rinderpest viruses and subsequent challenge with measles virus will complete the study.



Three strains of rinderpest virus passaged in tissue cultures have been reduced in virulence for cattle. All strains tested have been shown to share common antigenic factors. Some such strains may be useful as immunizing agents.

Although some progress has been made in electron microscopy studies, the virus particle of rinderpest has not yet been identified. It has been found that the virus is unstable in butanol and other lipid solvents. The virus was readily precipitated by centrifugation, but after being pelleted it could not be fully resuspended. Thus, purification procedures employing lipid solvents and pelleting have not been applicable. This ruled out, for the present at least, the possibility of using lymph nodes as a source of virus because of their relatively high content of impurities. Progress has been made, however, in concentrating and purifying tissue-culture virus. When the virus was found to withstand high salt concentration, it was possible to zone it by centrifugation in cesium chloride density gradients with nearly complete recovery of infectivity, 100-fold concentration, and the elimination of much debris. Such material after dialysis to remove salt, revealed a few virus-like particles, approximately 100 millimicrons in diameter, but they were too few to permit correlation with infectivity. The virus was concentrated about 100-fold by dialysis against polyethylene glycol, and its infectivity was unaffected by polyethylene glycol.

In 1962 relationships of the viruses of human measles, canine distemper and rinderpest were studied. Cattle injected with measles or distemper virus were not protected against challenge with rinderpest virus, although distemper antibodies were found in cattle injected with that virus. Each of the viruses elicited antibody response in an alien host. Puppies injected with either measles or rinderpest virus were protected against distemper.

Work was continued on the attenuation of the Kabete strain of rinderpest virus in tissue cultures. The modified virus retained sufficient antigenicity to immunize cattle, and in limited studies, it produced only limited clinical response. This strain of virus was inactivated at pH 2 and 12; but at pH 3, the virus remained viable for 5 minutes, and at pH 11, for 20 minutes. Virus-cell relationships were studied by electron microscopy. From this work, it is apparent that rinderpest virus develops in the mitochondria of infected bovine kidney cells in cultures.

Cross-immunity studies were made with one strain of bovine virus diarrhea and rinderpest. Calves recovering from experimental reinfection with virus diarrhea were susceptible to challenge with rinderpest virus, and serum from animals with virus diarrhea did not cross-react with rinderpest virus. Calves experimentally immunized against rinderpest, did not develop antibodies to virus diarrhea virus, and such animals were susceptible to challenge with virus diarrhea virus. These results indicate lack of serological or immunological relationship between the two viruses.

Q. Transmission of foot-and-mouth disease virus in semen.

In 1961 guinea pigs, bulls and heifers were used in preliminary studies. Guinea pigs and steers were used for infectivity studies. Type A, strain GB, FMDV was used in the studies in guinea pigs and types A, strain 119, and O-M11 were used in cattle.

Virus was found in the urine, testicles, vas deferens and epididymus of male guinea pigs 48 hours after inoculation via the metatarsal pads, but was not found in the seminal vesicles. The titer of virus in the vas deferens was higher than that observed in the other tissues and fluids. Virus was detected in the testicles 24, 48 and 72 hours after inoculation. Female guinea pigs showed lesions of FMD 120 hours after instillation of virus in the vagina or rubbing on the vulva. Other female guinea pigs failed to develop FMD when virus, diluted 1:10 with milk or egg yolk semen extenders, was instilled into the vagina. Control animals inoculated with diluted virus intradermally in the foot pads developed lesions within 24 hours. In limited trials, urine from infected male guinea pigs produced lesions when inoculated into the foot pads, but failed to produce FMD infection when instilled in the vagina of susceptible guinea pigs.

Two bulls were inoculated on the tongue with Type A, strain 119, FMDV and slaughtered 24 hours later, when signs and lesions of infection were evident in only one of the two animals; however, both developed viremia (titers of  $10^{4.8}$  and  $10^{4.5}$  ID<sub>50</sub>/ml, respectively). The testicles, epididymus and lining of the bladder from both animals contained infective virus. Virus was not found in the seminal vesicles or lining of the urethra from either bull, however, infective virus was demonstrated in the vas deferens from the bull which developed clinical signs of FMD prior to slaughter.

Heifers in oestrus and not in oestrus were used. The virus was prepared from infected bovine tongue tissue in various suspending fluids. Approximately 3.0 ml. of virus suspension was instilled in the vagina at a depth of about 12 inches, using precautions to prevent contamination of the vulva and surrounding area. Of two heifers receiving a bovine semen-virus preparation, one, which had been in oestrus 4 days prior to vaginal exposure, developed FMD within 4 days. The heifer not in oestrus did not develop clinical evidence of FMD and was susceptible to subsequent inoculation. Four heifers received the virus diluted with egg-yolk extender. One of three heifers in oestrus at time of exposure developed clinical FMD. The other two heifers remained free of evidence of infection and were susceptible to a subsequent inoculation. One heifer not in oestrus at the time of vaginal exposure developed signs and lesions of FMD 5 days later. Two additional heifers not in oestrus developed FMD within 4 days after vaginal exposure with 10 per cent suspension of Type O, strain M-11, virus diluted equally with bovine semen.

In 1962 semen samples were collected at various intervals after inoculation from 8 FMD-infected bulls by use of an electroejaculator. Urine and blood samples were also taken. Infectivity studies were conducted in mice and steers. FMDV (A-119 or O-M11) was found in the semen and urine of bulls as early as



12 hours and in blood as early as 6 hours after inoculation. During convalescence, virus was found in semen and urine for as long as 7 days and in the blood for as long as 4-3/4 days after inoculation. The titer of the virus in the semen usually was higher than in urine and sometimes higher than in the blood. The pH of the urine usually shifted from alkaline to acid about 2 days after inoculation and then returned to alkaline in about a week as the bulls recovered from acute infection. The pH of semen varied, but remained nearly neutral throughout the course of the disease.

R. Survival of foot-and-mouth disease in meat and meat products.

In 1961 studies previously reported demonstrated that FMDV may be found in lymph nodes, hemal nodes, bone marrow and large blood clots in carcasses of infected animals ripened at 4 C for 72 hours. The studies were extended to determine if virus also could be detected in bone marrow and lymph nodes of animals slaughtered at times after the initial stages of infection.

Virus was demonstrated in fresh rib bone marrow of steers for as long as 3 days after inoculation with FMDV A-119. Virus was not found in bone marrow of steers slaughtered 4, 5 and 9 days after inoculation with FMDV A-119, nor was virus found in bone marrow of a steer 7 days after inoculation with FMDV type SAT-3.

All 7 types of FMDV were used to determine the length of time after inoculation that the various viruses could be detected in lymph nodes. The number of days after inoculation that the various types and strains of infectious FMDV survived in lymph nodes of infected steers was as follows: 9 days for O-M11, C-149 and Asia 1; 11 days for A-119 and SAT-3, and 13 days for SAT-1 and SAT-2. However, on the 13th day post-inoculation, even though the test steers did not become clinically ill from the material inoculated from A-119 and C-149 infected donors, some of the test steers were sufficiently immunized by the material to resist subsequent challenge and had significant virus-neutralization indices, 3.7 and 5.5, respectively.

The titer of FMDV in the head and body lymph nodes of infected donor steers was compared on various days after inoculation, using 3 strains of FMDV (SAT-1, O-M11 and A-119). As might be expected, since the steers were infected by tongue inoculation, the titer of the virus was usually slightly higher in the head lymph nodes (mandibular primarily) than in the body nodes. For the first 3 days after inoculation the virus titers in the lymph nodes varied from 1.3 to 4.5, with an average of 3.2 (bovine ID<sub>50</sub> or mouse LD<sub>50</sub> per ml. based on 15 titrations). At the 7th, 8th and 9th days after inoculation, the titers varied from 1.3 to 3.0, with an average of 2.4 for 5 observations. No virus was demonstrated in 11 other samples tested. Virus titers as high as 5.6 have been obtained from the blood of steers on the third day after inoculation with FMDV A-119, and 7.8 or higher from infected tongue tissue, while the titer in lymph nodes has not exceeded 4.5

In 1962 ground meat, composed of lymph nodes and muscle tissue, in a ratio of 1:10, was prepared from FMD-infected steer carcasses stored at 4 C for 72 hours. The ground meat was stored at 4 C, unsalted and salted (4% NaCl). When fluids were expressed from unsalted meat, FMDV was found as long as 11 days, but could not be detected in salted meat stored for more than 4 hours. However, when the lymph-node fragments were sorted from the ground meat and tested, virus was found in salted meat for as long as 17 days.

The titer of FMDV in lymph nodes declines during storage; 34 samples gave an average titer of  $10^{3.8}$  (fresh) and about  $10^{1.6}$  after 9-10 days storage. FMDV in lymph nodes taken during the pre-clinical stage of the disease apparently does not survive as long during storage as virus in lymph nodes taken after the disease is well established.



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PARASITES AND PARASITIC DISEASES OF CATTLE  
Animal Disease and Parasite Research Division, ARS

Problem. The cost of parasitic diseases to the cattle industry of the United States is estimated to be in excess of \$400 million annually. Disorders caused by parasites are ubiquitous, generally insidious and often overlooked entirely. Diagnosis is difficult and successful treatments for many of these diseases are not available. Moreover, management practices to avoid spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling or eradicating parasitic diseases so as to provide for healthy cattle, insure adequate supplies of parasite-free beef for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a more prosperous agriculture and the national economy.

USDA PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, parasitologists, pathologists and veterinarians engaged in both basic and applied studies directed to the development of measures for the solution to the high and extremely costly incidence of parasitism in cattle. Research is being conducted on parasitic diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 17.7 professional man-years. This effort is divided among subheadings as follows:

Ecological Factors Influencing Nematode Development 1.1 at the Regional Animal Parasite Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Effects of Mixed Helminth Infections 2.0 at the Regional Animal Parasite Laboratory, Auburn, Alabama.

Acquisition of Parasites from Pastures 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Effect of Pasture Mixtures and Pasture Management on Control of Internal Parasites 1.5 at the Regional Animal Parasite Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Winter Coccidiosis (Bloody Scours) 1.0 at the Regional Animal Disease and Parasite Laboratory, Logan, Utah, and under a cooperative agreement with the Montana Agricultural Experiment Station, Bozeman.



Influence of Diet and Nutrition of Cattle on Roundworm Parasitism 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Artificial Propagation of Protozoan Parasites 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Host-Parasite Relationships of Coccidia 1.0 at the Regional Animal Parasite Laboratory, Auburn, Alabama.

Ecology and Immunology of Lungworms 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Clinical and Physiological Aspects of Roundworm Parasitism in Cattle 0.1 at the University of California, Davis under a cooperative agreement with the USDA.

Investigations of Trichomonad Parasites 1.0 at the Regional Animal Disease and Parasite Laboratory, Logan, Utah, and under a cooperative agreement with the Utah Agricultural Experiment Station, Logan.

Host-Parasite Relationship of Intestinal Worms Cooperia spp. 2.0 at the Regional Animal Parasite Laboratory, Auburn, Alabama.

Anaplasmosis 4.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland, and through memoranda of understanding and other arrangements in cooperation with State Experiment Stations in California, Illinois, Louisiana and Nevada, the State Veterinarian of Tennessee, the USDA Entomology Research Station, Kerrville, Texas, the Delta Branch Experiment Station, Stoneville, Mississippi, and a large cattle ranch in Virginia and in Wyoming.

Investigations on Anaplasmosis, Piroplasmosis and Babesiellosis of Cattle, are under way through a PL 480 Grant, at the School of Veterinary Faculty, Montevideo, Uruguay.

Investigations on the Pathogenesis of Lesions Produced by the Leech, Limnatus nilotica are under way at the Hadassah-Hebrew University Medical School, Israel, under a PL 480 grant.

#### RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 21.7 professional man-years divided among subheadings as follows: Ecological factors influencing nematode development 2.4; mixed helminth infections 4.4; acquisition of parasites from pastures 3.9; winter coccidiosis (bloody scours) 1.0; influence of diet and nutrition of cattle on roundworm parasitism 0.9; clinical and physiological aspects of roundworm parasitism in cattle 1.1; anaplasmosis 6.9. Nine southern and four western States and

the USDA cooperate in regional research (S-21, Gastrointestinal Parasites of Ruminants, and W-35, Nematode Parasites of Ruminants) to determine the Ecological Factors Influencing Nematode Development. The two regional projects on internal parasites of ruminants (S-21 and W-35) provide a basis for cooperation on the project Mixed Helminth Infections. The factors which influence the Acquisition of Parasites from Pastures by cattle are being studied in the South (Regional project S-21). Georgia, Louisiana, and Puerto Rico are studying conditions such as moisture on grass blades, sunlight striking the blade, height and type of grasses. Studies conducted in Mississippi include the effect of different seasons, the type of seasonal grasses and soil sterilants on parasite larvae. Arkansas, Georgia, Louisiana, and Texas are cooperating in regional research (S-21) to evaluate factors of parasite control. Examples are management, rotation, stocking of pastures, supplemental feeding, water, soil types, drainage and shade. Georgia, Mississippi and Oregon are determining which are the best grasses or mixture of grasses for parasite control. Studies in cooperation with the USDA are in progress in the North Central Region and the Western Region on contributing conditions for Winter Coccidiosis (bloody scours). The evaluation of specific food elements, carbohydrates, fats, minerals, proteins and vitamins, are being studied in the North Central Region and Southern Region (S-21) in cooperation with the USDA to determine the Influence of Diet and Nutrition on Roundworm Parasitism. The Southern Region (S-21) and Western Region (W-35) are cooperating with the USDA to determine the Clinical and Physiological Aspects of Roundworm Parasitism in Cattle. Research studies on Anaplasmosis are in progress in the north central, southern, and western regions in cooperation with the USDA.

Industry and Other Organizations especially chemical companies, are engaged in research on the formulation of compounds and the exploration of chemicals that may be used safely as parasiticides. Most of the companies engaged in this kind of research utilize their own personnel, facilities and funds. The efforts and results of the work are generally considered as confidential since the ultimate goal of the companies is to produce saleable products. It is estimated that approximately 50 professional man-years are devoted to the work.

## REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

### A. Ecological Factors Influencing Nematode Development.

In 1961 at the Animal Disease and Parasite Research Division's (ADP) Regional Animal Parasite Laboratory, Auburn, Alabama, the research showed that eggs are laid by Cooperia oncophora in various stages of development and their hatching times are correspondingly varied. Measurements, stages of development and appropriate comparisons with C. punctata and C. curticei have been made. The shortest prepatent period was 17 days. The first 18 feet of the small intestine was found to harbor the majority of worms. The patent period of a single infection may last as long as 8 months.



In a study on immunity to the ruminant parasite, Trichostrongylus colubriformis, subcutaneous injection of guinea pigs with artificially exsheathed infective larvae, intraperitoneal injection with the metabolic excretions and secretions of artificially exsheathed larvae, and oral inoculations with 5,000 normal infective larvae, failed to afford any protection to the guinea pigs against subsequent oral challenge with 40,000 infective larvae.

Cooperative work at Experiment, Georgia, under a memorandum of understanding, indicated, in preliminary studies, a reduction in the number of third-stage larvae of various cattle and sheep nematodes proportional to the increase in number of viable spores of Bacillus thuringiensis var. thuringiensis Berliner.

In 1962, at Experiment, Georgia, under the auspices of the ADP Laboratory at Auburn, work on the ecological factors influencing nematode development, but using guinea pigs and rabbits for the tests, revealed a markedly lower number of adult T. colubriformis was recovered from guinea pigs infected with larvae cultured at 10°C than from three other groups of guinea pigs infected with larvae reared at 15, 25, or 32°C. The hosts infected with larvae cultured at 32°C had a much higher number of larvae than those from the other three groups. However, another test using larvae of T. axei to infect rabbits showed no difference between the number of worms recovered from the rabbits infected with larvae reared at 10 and 25°C, but a lower number of larvae was recovered from rabbits infected with larvae cultured at 32°C.

#### B. Effects of Mixed Helminth Infections.

In 1961, at the Regional Animal Parasite Laboratory, Auburn, Alabama, it was found that 5.5 to 6 months-old grade Jersey calves, administered from 200,000 to 700,000 infective larvae of Cooperia oncophora, developed anorexia and an enteritis during the patent period of infection. Three groups of infected calves made average total weight gains ranging from 6.5 to 20.5 pounds less than that made by the controls; however, the differences were not significant. Blood physiology was not affected by the infections.

In 1962 this work was discontinued early in the year.

#### C. Acquisition of Parasites from Pastures.

In 1961 at the Beltsville Parasitological Laboratory (BPL), a study was made of the development of infective larvae of gastro-intestinal parasites of cattle, their migration onto herbage, and their duration thereon following deposition of feces containing eggs of these parasites at different times of the year. In general, conditions for development, migration onto the herbage, and survival were best in early Fall and in the Spring when there was an abundance of available moisture, and they were poorest in late Fall, when it was cold, and in Midsummer when it was too dry. These findings are in agreement with earlier indications of larval development and survival obtained from the numbers of worms recovered from calves that grazed off test plots.

In 1962 at the Beltsville Parasitological Laboratory, studies were continued and indicated that rotational grazing of pastures by bovines infected with gastro-intestinal nematodes apparently did not reduce levels of parasitism or improve the performance of infected animals in an experiment carried out in 1961. Two test groups initially were artificially exposed to infection equally and simultaneously. During the experiment one of the groups grazed three 1-acre pastures rotationally. The other grazed continuously on a single 3-acre pasture. The initial and each subsequent grazing period on each smaller pasture was 2 weeks, so each 1-acre plot was vacant for 4 weeks prior to each re-grazing of it. Three cycles of rotation were completed from early July to early November, when the experiment ended and the cattle were necropsied. The worms recovered at necropsy have not yet been counted, but worm-egg counts during the test indicated no substantial difference in the worm loads of the two groups.

Initial infection with the beef measles worm (Cysticercus bovis) conferred on cattle in a recent test a strong resistance (acquired resistance) to reinfection with this larval tapeworm. The results of tests to explore the possibility that age may be a factor in initial susceptibility to this parasite suggest that adult animals, with the possible exception of ones more than 5 years old, can be at least as susceptible as calves and that individual variation in susceptibility occurs.

#### D. The Effect of Pasture Mixtures and Pasture Management on the Control of Internal Parasites of Cattle.

In 1961, at Experiment, Georgia, a second year feeding trial was conducted under the supervision of the Regional Animal Parasite Laboratory at Auburn. The findings reported for the first year's grazing trials were not statistically significant. The second year test was completed studying the effect of rotational grazing on different types of pasture mixtures on the level of parasitism in beef yearlings. One of 2 plots planted with temporary winter pasture was divided into 4 plots and grazed on a four-way rotational system. A third plot was also divided into four plots, each of which was planted with a different forage mixture and rotationally grazed. For the second year, the steers on the winter temporary pasture rotationally grazed harbored more worms (34,827) and exhibited a lower average daily gain (ADG) than those animals grazed continuously on the same type of forage (22,674 and 2.09 lbs.). The lower number of parasites recovered from the rotationally grazed pasture mixtures (17,149) was probably due to the excessive growth of these forages, which also reduced the ADG (1.84 lbs.), although the average stocking rate was lower than those from the other lots.

In 1962, the studies were continued at Experiment, Georgia, where the third-year test was completed in an attempt to study the effects of rotational grazing on different types of pasture mixtures on the level of parasitism in beef yearlings. One of the two lots planted with temporary pasture was divided into four plots and grazed on a four-way rotational system. A third lot was also divided into four plots, each of which was planted with a different forage mixture and rotationally grazed. The animals from the



continuously grazed pasture had less worms (15,109) and higher ADG (3.00 lb.) than those from the two rotationally grazed pastures - 41,229 worms and 2.34 lb. from the winter temporary, and 22,299 worms and 2.15 lb. from the pasture mixture. Rotation of grazing required higher stocking rate for proper pasture utilization, which may be responsible for increased parasitism.

#### E. Winter Coccidiosis (Bloody Scours) of Cattle.

In 1961, at the Animal Disease and Parasite Laboratory, Logan, Utah, fifteen calves were used in one experiment to investigate the possible transmission of passive immunity to coccidiosis caused by Eimeria bovis through intraperitoneal injection of concentrated serum globulin, and to observe the effects of intraperitoneal injections of sporulated oocysts, or of merozoite-mucosa emulsion on the development of clinical coccidiosis. The intraperitoneal injection of sporulated oocysts resulted in the development of mild coccidiosis sufficient to cause development of enough immunity to resist per os inoculations with 1.4 million sporulated oocysts. Intraperitoneal injections with concentrated serum globulin, merozoite-mucosa emulsion, or distilled water failed to prevent coccidiosis when the animals were given 1.4 million sporulated oocysts per os. There was no passive transfer of immunity nor alterations of the serum proteins related to any of the injections.

In another experiment sixteen yearling steer calves were used to determine how long immunity to coccidiosis persisted. Approximately 1 to 1-1.3 years after their last experimental inoculation with sporulated oocysts each of 16 calves was inoculated per os with 1.4 million sporulated oocysts. Only calves which had not previously been inoculated developed severe symptoms of coccidiosis. Severe changes in the serum protein accompanied or followed the occurrence of coccidiosis in susceptible calves. More pronounced changes in the beta and gamma globulin fractions were observed in these older calves than in the young calves. One calf, immunized about 9 months earlier and treated with sulfa drugs, developed unusually severe reductions in serum protein during the period when the susceptible calves were exhibiting severe symptoms of coccidiosis. This calf showed none of the usual symptoms seen in the other calves. This may indicate that the effects of coccidial infections are manifested in ways other than the usual diarrhea, bleeding, etc.

Thirty newly weaned calves averaging about 350 pounds each, were used to determine the effect of intraperitoneally or intramuscularly injected oocysts on the alteration of serum proteins and the development of immunity to coccidiosis. Calves injected intraperitoneally with sporulated oocysts appeared to develop resistance to per os inoculation but intramuscular injections with sporulated oocysts produced little or no immunity. Intraperitoneal and intramuscular injections with unsporulated oocysts failed to elicit the development of immunity. All of the 30 calves carried light natural infections at the time of inoculation, but per os inoculation with 300,000 sporulated oocysts produced severe effects which were sufficient to kill one calf.

In 1962 at the Logan Laboratory, the research work on winter coccidiosis was continued. No significant differences were observed in the susceptibility to coccidiosis, Eimeria bovis, infections in nursing and bucket-fed new-born calves. Uninoculated nursing control calves quickly became infected when penned with calves that had been inoculated with coccidia, but the infection was less severe. The administration of whole citrated blood intravenously to calves during the severe stage of coccidial infection, appeared to increase hemorrhage. Severe infections did not significantly alter the levels of hemoglobin, hematocrit and blood glucose, or the total serum protein.

Calves given single intraperitoneal injections with 1,000,000 sporulated oocysts of E. bovis developed clinical coccidiosis and one of eight died. Those surviving exhibited a strong immunity to reinfection. Four calves given multiple alternate day injections of 200,000 sporulated oocysts for five injections, exhibited symptoms over a longer period than calves given a single injection. One of the four calves died, but the three survivors exhibited stronger immunity to reinfection than did any of those in the other groups.

Infection was not manifested in any of another group of calves that were injected intraperitoneally with sporulated oocysts of E. bovis. This may indicate that the former injections were given into the intestinal tract instead of into the peritoneal cavity. Two calves injected intraperitoneally with x-ray irradiated oocysts that had been exposed to 60,000 r by a Westinghouse Quadrocondex machine did not develop signs of infection, or any immunity. Two calves given oral inoculations with irradiated oocysts showed a mild infection but exhibited immunity to reinfections.

There were no changes in the serum electrolytes, potassium or sodium, during prepatent or patent periods, or significant changes in the sera of those calves that survived the infections. Immediately before death, from coccidiosis, the blood serum potassium was elevated to 7-8 mEq/l while the sodium levels were reduced to 90-100 mEq/l.

In 1962, at the Montana Veterinary Research Laboratory, Agricultural Experiment Station, Bozeman, under a cooperative agreement with the USDA, research work was conducted on winter coccidiosis. Observations were made on the comparative morphology and sporulation time of Eimeria ellipsoidalis, E. bovis, E. auburnensis, E. cylindrica, E. zurnii and E. brasiliensis. An attempt was made to provide criteria for differentiation of these species, and to clarify some of the apparent inconsistencies in the taxonomy of the cattle coccidia. A progressive increase was shown in oocyst length and width from E. cylindrica through E. ellipsoidalis, E. bovis, and E. brasiliensis. Sporulation time at 20 and 30°C was also distinct.

The occurrence of coccidial strains resembling E. cylindrica either morphologically or with respect to sporulation time, but differing markedly in other respects, was demonstrated in naturally infected beef calves. It is important that valid criteria be provided for differentiating cattle coccidia, since the pathogenicity of the various species differs greatly.



#### F. Influence of Diet and Nutrition of Cattle on Roundworms.

In 1961, at the Beltsville Parasitological Laboratory, Beltsville, Maryland, two comparable groups of calves were fed different levels of the same diet. Half of each group was infected with equal numbers of the same kinds of gastrointestinal helminth parasites. The infected calves on the lower level of feed consumption became more heavily infected, and, relative to their respective controls were, in general, more adversely affected by the parasitism than those on the higher level. Replication is necessary before generalization is justified.

In 1962, these studies were continued at the Beltsville Parasitological Laboratory. The results showed the efficiency of feed utilization by calves on two different levels of feeding was markedly reduced by moderate infection with gastrointestinal nematodes. Efficiency was also affected by the level of feeding and was greater at the higher level. However, the reduction in efficiency caused by the parasitism was about 8 times the difference due to feeding level. Calves on the higher intake were less severely affected by the parasitism than those on the lower level. In mild infections the higher feeding level only slightly enhanced the ability of the calves to cope with the infections and efficiency of feed utilization was affected about equally by parasitism and feeding level.

#### G. Artificial Propagation of Protozoan Parasites.

In 1961 at the Beltsville Parasitological Laboratory, work was continued to develop a defined medium for the in vitro cultivation of Histomonas meleagridis, the causative agent of blackhead. This parasite was successfully propagated for the first time free of demonstrable bacteria in modified tissue culture media. Bacteria from the ceca of turkeys, heretofore considered important and routinely used in cultures of Histomonas, were successfully replaced by a variety of fresh hamster tissues enriched with metal ions, without loss of infectivity of the parasites. Histomonads grown in these tissue-containing media were capable of infecting young chickens when inoculated by rectum, whereas organisms grown in media enriched with bacteria from the ceca of turkeys were unable to do so.

Histomonads were grown in media devoid of cream, but containing cholesterol, cholesterol esters or a commercial steroid preparation which demonstrated for the first time that one of the growth factors for Histomonas is a steroid. It was found that certain fatty acid esters of cholesterol promote good histomonad growth, thereby indicating that another growth enhancing compound is probably a lipid.

Primary requisites to artificial cultivation of parasites are (1) freedom from contaminating organisms, and (2) an adequate supply of the infective stages of the parasite. With regard to coccidia, both these problems were solved.

Sterile suspensions of Eimeria acervulina were obtained with antibiotics. Sporocysts were released from oocysts by aseptic grinding with a mortar and pestle, and excystation of sporozoites from sporocysts was produced by treatment with sterile trypsin and chicken bile. A 1 cc suspension containing as many as 3 million motile sporozoites--more than enough--was obtained by use of trypsin and chicken or turkey bile. Excystation occurred within 5-10 minutes and 90-95 percent excysted within 1 hour.

In 1962 research workers at the Beltsville Parasitological Laboratory reported that cholesteryl palmitate and stearate were used successfully as replacements for cream in artificial cultivation of the protozoan parasite, Histomonas meleagridis. Growth factor(s) for this parasite, provided by certain bacteria normally associated in the host, and grown in nutrient broth, appear to be intra-cellular. This factor(s) can be inactivated by subjecting the bacteria to temperatures above 56°C and below freezing, but can be regenerated by returning the bacteria to their normal culture temperature.

Bacteria grown in a variety of media other than nutrient broth have failed to sustain histomonad growth. A modification of a commercial tissue culture medium, known as "199", in which histomonads will grow after the addition of cream from cow's milk, was found capable of supporting histomonad growth even after being frozen for more than 2 months.

#### H. Host-Parasite Relationships of Coccidia.

In 1961, at the Regional Animal Parasite Laboratory, Auburn, Alabama, studies on histochemical staining revealed (a) PAS reactions were positive in E. alabamensis in oocysts in tissues and in macrogametocytes. Heavily parasitized host cells, as well as mature schizonts, ceased to be PAS positive; (b) Glycogen was found in macrogametocytes of E. alabamensis and E. zurnii, as well as in some mature schizonts of E. zurnii and E. bovis; (c) Collagen fibers and keratin were found in the outer membranes covering the macroscopic schizonts of E. bovis; (d) polysaccharides, DNA and protein were studied in macroscopic schizonts of E. auburnensis and during various times in the life cycle of E. ahsata in sheep.

An acid stain by Gray, et al, using Celestine Blue B, ferric alum, glycerol and sulfuric acid was found to be the equivalent to iron hematoxylin stain, even though only 1 minute is required for staining. Intermediate stages of coccidia in sections of cattle intestines were stained by this method.

The Lotze method of excysting oocysts of coccidia in vitro by using overnight exposure to lipase (steapsin) solution and then additional exposure to fresh bile was confirmed at the Regional Laboratory. Living sporocysts and sporozoites of E. ahsata, freed by this method, were measures.

In 5 of 6 calves inoculated with oocysts of Eimeria bukidnonensis, the pre-patent period was from 11 to 16 days and the patent period from 4 to 6 days. Three calves showed signs of clinical coccidiosis.



Oocysts of Eimeria auburnensis, stored in 2% potassium dichromate solution, were still viable after 3.5 to 4 years. Schizonts of E. auburnensis, ranging up to 250 by 100 $\mu$  were found in the lower half of the small intestine of a calf killed on the 12th day after inoculation. Most were embedded in the mucosal layer instead of in the center of the villi as is common in E. bovis.

In 1962 studies were continued at the Auburn Laboratory. The results of several tests were (a) Schizonts were found in the middle and posterior third of the small intestine of calves killed 12 and 14 days after they had been inoculated with pure cultures of oocysts of Eimeria auburnensis. The schizonts ranged from 78 $\mu$  to 250 $\mu$  long by 78 $\mu$  to 150 $\mu$  wide. (Sample mean 92 $\mu$  by 139.9 $\mu$ ). They were usually located deep in the lamina propria near the muscularis mucosae instead of in the villi where most schizonts of E. bovis are found. The schizonts of E. auburnensis resemble the previously described large microgametocytes of this species, but were distinguishable morphologically and by histochemical stains. The microgametocytes were much larger than previously reported; one measured 91 $\mu$  by 287.5 $\mu$ .

(b) Calves were killed 4, 11, 15, and 25 days after inoculating with Eimeria bukidnonensis. Studies of the tissues revealed sporozoites in sections of small intestine 48 ft. above the ileocecal valve, at 4 days. At 11 days, a young schizont was found at C + 12. Nothing was found at 15 but, at 25 days, an oocyst was located at C + 1.

(c) The following mature endogenous stages of Eimeria zurnii, E. alabamensis, and E. bovis, coccidia of cattle, were periodic acid Schiff ("PAS") positive: merozoites, microgametocytes, traces of cytoplasm and plastic granules of macrogametes and oocysts. Immature forms of the same stages were usually PAS negative.

(d) Celestin Blue B, Acid Fuchsin, and Orange G gave very good contrasting colors to various endogenous stages of Eimeria zurnii in sections of cattle intestines.

(e) In two tests on excystation of oocysts of Eimeria ahsata, a solution of chenodesoxycholic acid, a purified bile extract, did not induce liberation of sporozoites. Sodium desoxycholate, 0.5%, following lipase exposure, released 20 percent.

#### I. Ecology and Immunology of Lungworms.

In 1961, at the Beltsville Parasitological Laboratory, research showed that double vaccination of calves with x-irradiated larvae of the cattle lungworm usually offered some degree of protection against challenge exposure with this parasite. The immunization was accomplished with larvae exposed to 40,000 roentgens at 4 different rates - 100, 200, 400, and 1,200+ roentgens per minute. The larvae exposed at the lowest rate were reared and irradiated by a commercial firm. The rate of x-ray application did not materially affect the invasive powers of the larvae in mice and guinea pigs. However, challenge exposures produced the least pulmonary distress in calves given orally larvae

irradiated at the lowest rate. Also, these calves eliminated negligible numbers of first-stage larvae, had the least amount of lung damage at post-mortem, and no worms were grossly observed at necropsy.

In 1962, at the Beltsville Laboratory (BPL) it was found that double oral vaccination with x-irradiated cattle lungworm larvae conferred resistance to infection with this parasite on 2 of 4 pairs of test calves. At necropsy about 1 month after challenge exposure to infection with normal larvae, each of these pairs yielded fewer worms than any of 4 control calves. One calf was vaccinated with larvae irradiated at a rate of 100 r/min., the other with larvae irradiated at 1,200+ r/min. The remaining two pairs of calves were vaccinated with larvae irradiated at intermediate rates. One of these pairs was highly susceptible, the other possibly slightly resistant to infection on challenge. An adequate explanation for these differences in results is not presently available.

Oral vaccinations with larvae of a sheep lungworm appeared to be only partially successful in immunizing calves against the cattle lungworm.

#### J. Clinical and Physiological Aspects of Roundworms.

In 1961, at the School of Veterinary Medicine, University of California, Davis, under a cooperative agreement with the USDA, Ruelene, an organic phosphate with excellent anthelmintic properties, was found to be of greatest value when administered orally. When poured on the back, or injected intraperitoneally in cattle, anthelmintic activity was unsatisfactory. Iodine-free phenothiazine was found to have a slightly greater anthelmintic action than N.F. phenothiazine. The increased activity, however, was too small to account for the difference previously found between purified and N.F. phenothiazine.

In 1962, cooperative research at the California laboratory, showed that experimental Micellar phenothiazine is no more effective than good grade National Formula, but is much better than older commercial preparations. To obtain satisfactory anthelmintic action with phenothiazine, it is necessary to consider specific surface, purity as well as total dosage. The use of dosage recommendations based on dose area per kilogram body weight will probably give satisfactory results. The interaction of phenothiazine and certain organophosphates when utilized as anthelmintics was found to be additive rather than synergistic.

Hypoalbuminemia in heavily parasitized cattle was found to be due to reduced synthesis and only in terminal cases was an increased catabolism observed. Preliminary ferrokinetic studies in similar animals indicated that the anemia may result from bone marrow by poplasia and marrow hemolysis.



#### K. Investigations of Trichomonad Parasites.

In 1962, at the Regional Animal Disease Laboratory, Logan, Utah, limited research was conducted on the new project since it was necessary to acquire several cultures of Trichomonas foetus from different areas of the United States. One culture was obtained from England. Rabbits were used for antibody production since they are not known to harbor T. foetus. However, it was found that rabbits do not tolerate a long series of injections very well. Best results were obtained for separation of rabbit-serum fractions by unidimensional starch-gel electrophoresis using a voltage drop of 6 volts per centimeter at room temperature over a period of 20 - 22 hours.

In 1962, at the Utah Agricultural Experiment Station, Logan, under a cooperative agreement with the USDA, research workers isolated a pentatrichomonad from the rumen and cecum of calves. The forms of the pentatrichomonads from both locations appear to be identical.

#### L. Host-Parasite Relationship of Intestinal Worms Cooperia spp.

In 1962, at the Regional Animal Parasite Laboratory, Auburn, Alabama, progress was made on this, a newly instituted, project of research. It was found that calves 6 to 7½ months old, were severely affected by oral inoculation with 350,000 Cooperia pectinata infective larvae. The infected calves made an average weight gain of 3.8 pounds while the controls averaged 45.3 pounds. The clinically affected calves developed a pronounced hypoglycemia concomitant to the period of anorexia. The pathogenicity of this species is essentially as severe as that of the related form Cooperia punctata, and both species are much more pathogenic than C. oncophora.

The life history of Cooperia pectinata, an intestinal worm of cattle, is direct, requiring from 14 to 17 days to develop from the ingested infective larva to the sexually mature adult. Its rate of development is intermediate between that of C. punctata and C. oncophora. Attempts to produce hybrid nematode crosses between C. oncophora and C. pectinata were initiated. Fourth-stage female C. oncophora with fourth-stage male C. pectinata and fourth-stage male C. oncophora with fourth-stage female C. pectinata were successfully transferred to the small intestines of laparotomized helminth-free calves. Eggs in these calves' feces developed to infective larvae in culture. Photomicrographs were made of a male C. pectinata and a female C. oncophora in copula. The progeny are being studied. Chromosome preparation and staining techniques have been developed for use in this study.

A pilot study was made of the histochemistry of Obeliscoides cuniculi and of Ostertagia ostertagi and of the histochemical pathology of infection by worms of the latter species in calves. Histochemical techniques used have been Best's carmine stain, the periodic acid-Schiff reaction, the ferric mannitol technique, the Alcian blue technique, the iodine method of Nielsen, Okkels, and Stockholm (loc. cit.), and the calcium-cobalt method of Gomori for phosphomonoesterase I.

Best-positive material (glycogen) was found in the intestinal walls, ovary, and musculature of the body wall of Obeliscoides cuniculi. PA/S-positive material was detected in the cells of the digestive tract, musculature and chords of the body wall, gonads, and pseudocoelom of these worms. After treatment with malt diastase, Best-positive material is completely absent, and PA/S-positive material is absent from gonads, intestinal cells (but not the surface of the lining membrane), and musculature of body wall. Other histochemical techniques were not used with Obeliscoides.

In vitro tests using the Goodey skin penetration technique indicated that T. axei and T. colubriformis larvae were unable to effect skin penetration. Adult worms were recovered from the intestines of rabbits exposed to cutaneous applications of T. axei and T. colubriformis. The low yield of adult worms indicates that the larvae did not enter the rabbit percutaneously, but were probably ingested from the surface of the contaminated skin.

#### M. Anaplasmosis of Cattle.

In 1961, at the Beltsville Parasitological Laboratory, research workers reported immunity studies in cattle, using an adsorbed non-viable vaccine prepared from concentrated anaplasma-infected blood, resulted in prolonged serum antibody titers to anaplasma complement-fixing antigen. The vaccinated animals did not have appreciable immunity when exposed to low doses of infected blood.

Serologically suspicious cattle, revealed by the anaplasmosis complement-fixation test, in a large commercial herd, have been studied for anaplasmosis by inoculation tests into susceptible cattle. Two of 36 such animals (5.5%) were found to be infected. These and similar previous studies indicate the need for more research on the problem of causes of CF suspects and means for separating non-specific from specific reactions.

A series of experimental transmission trials with the Rocky Mountain wood tick (Dermacentor andersoni) have indicated that transovarian passage of the anaplasma agent in this tick is not a common finding. It was shown experimentally that infected male ticks could transmit anaplasmosis after a period of 108 days. The relative importance of these findings to actual spread of the disease in nature is still problematical, but the information obtained to date does suggest that the male tick may be responsible for considerable natural spread of tick-borne anaplasmosis.

The fluorescent antibody technic has been applied to studies on the agent of anaplasmosis. A variety of anaplasma forms, many possessing tail-like structures, have been observed in the blood of some infected animals. Filtration experiments, using plasma from acute cases of anaplasmosis, and Millipore filters of known pore size, have established the presence of an extra-cellular form of the anaplasmosis agent. Such plasma, when passed through filters of 0.65 micron and 0.3 micron average pore size, has been found to be infective for cattle.



Field trial studies at (1) Kerrville, Texas, indicate after 2 years that isolated clean offspring of carrier cattle have remained free of anaplasmosis without the use of insect control measures; (2) Evanston, Wyoming, indicate that when negative susceptible cattle are placed on tick-infested rangelands, that anaplasmosis re-infection of the animals and pastures by indigenous Rocky Mountain wood ticks has been of slight likelihood during the past year; (3) Stoneville, Mississippi, indicate that the use of an electric-eye, automatically operated walk-through sprayer, using synergized pyrethrum, can substantially reduce the losses and spread of anaplasmosis in an infected herd.

In 1962, research on anaplasmosis of cattle was continued at the Beltsville and Kerrville laboratories, with the following results:

Transmission trials using the Rocky Mountain wood tick, Dermacentor andersoni Stiles, failed to demonstrate transovarian passage of the etiological agent of anaplasmosis. Ticks of this species, collected from an infected area in Wyoming, did not transmit anaplasmosis when tested on known susceptible calves. A colony of these ticks was established and stage to stage (nymph to adult) transmission of anaplasmosis was accomplished. D. andersoni male ticks transmitted the disease after they were held for 197 days following feeding on an acute case of anaplasmosis.

The morphological variations of Anaplasma marginale Theiler, as observed in infected erythrocytes by electron microscopy and immunofluorescent methods, have been studied. The forms observed by both techniques included those with and without projections or sac-like structures. It was determined that the projection of the Anaplasma was a valid structure and not an artifact. Ticks feeding on highly parasitized blood were found to eliminate these structures in their excreta.

Serological studies were done to compare the standardized complement-fixation (CF) serum reaction of infected animals with reactions produced by serums in a capillary agglutination (CA) test. It was found that serums preserved with a low concentration of phenol as used in the CF test did not give satisfactory results in the CA test. Although there was close agreement between results of CF and CA tests, the comparative accuracy of the two testing methods was not definitely established. It was found that some CF antigens would agglutinate in the presence of immune sera from infected animals.

The causative agent of anaplasmosis was frozen by a slow-freezing technique but failed to survive prolonged cold storage even though it did survive freezing and storage at -60°C for a 24-hour period.

Experimental field control studies demonstrated a protective effect from feeding low levels of Chlortetracycline to susceptible cattle. The natural course of anaplasmosis in a dairy herd in Louisiana is being studied to evaluate the effect of the disease on milk production. Rocky Mountain wood ticks in Wyoming are being investigated as natural transmitters of anaplasmosis.

The anaplasmosis research herd, Kerrville, Texas. The objective set in 1958 for this project has been achieved by using the complement-fixation test to identify reacting animals, isolation of reactors from negative cattle by a wire fence, strict antisepsis in all handling of the animals, a minimum of arthropod control, the original reactor herd of 38 cows and a bull eventually replaced, retention of negative heifers of that herd, and by a negatively reacting herd of 46 females and a bull. Once isolation of the heifers was accomplished, there were no breaks with the disease among them.

Entomology Research Division cattle. Regular blood samples are obtained from the main herd and from cattle imported from other States for research purposes. The disease continues to spread among these cattle and clinically recognizable cases of anaplasmosis were rather frequent during the year. Since antisepsis is routinely practiced for the blood sampling, the transmission is accomplished either by arthropod vectors or some other operation.

In 1962, a project on Investigations on Anaplasmosis, Piroplasmosis and Babesiellosis of Cattle was initiated under a PL 480 grant to the School of Veterinary, Montevideo, Uruguay. No report on progress was submitted for the quarter ending June 30, 1962.

In 1962, a project on Investigations on the Pathogenesis of Lesions Produced by the local Leech, Limnatis nilotica, was initiated under a PL 480 grant to the Hadassah Medical School, Hebrew University of Jerusalem, Israel. No report on progress has been made.



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INFECTIOUS AND NONINFECTIOUS DISEASES OF SWINE  
Animal Disease and Parasite Research Division, ARS

Problem. Profitable swine production depends largely on the ability to control diseases. Swine diseases cause losses estimated at more than \$200 million annually. In order to control and eventually eradicate these diseases, a thorough knowledge of causes, diagnostic procedures, preventive procedures, and treatments is required. Although a great deal of excellent research has been and is being accomplished, a vast amount of research is still required to obtain this knowledge. At present, the causes of several important swine diseases are unknown or incompletely understood. Extensive fundamental research on swine diseases is essential to the welfare of the swine industry.

USDA PROGRAM

The Department has a long history of swine disease research. For example, research on hog cholera was initiated in 1884. Research on this and other important swine diseases is a continuing long-term program. Modern research techniques in the areas of biochemistry, biophysics, pathology, microbiology, pharmacology, physiology, and immunology, are being applied to swine disease problems. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 22.3 professional man years. This effort is divided among sub-headings as follows:

Hog Cholera 8.1 at the National Animal Disease Laboratory, Ames, Iowa, the Florida Hog Cholera Research Station, Live Oak, Florida, and under a cooperative agreement with the University of Illinois.

Atrophic Rhinitis 4.0 at the National Animal Disease Laboratory, Ames, Iowa.

Transmissible Gastroenteritis 3.6 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with Purdue University and the University of California, and a memorandum of understanding with the University of Illinois.

Erysipelas 3.6 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the Department of Biochemistry, Seton Hall College of Medicine and Dentistry, Jersey City, New Jersey.

Brucellosis 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

## RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 18.1 professional man years divided among sub-headings as follows: death losses in young pigs 5.6, hog cholera 3.4, atrophic rhinitis 2.0, transmissible gastroenteritis 2.7, erysipelas 0.7, brucellosis 2.4, other diseases (salmonellosis, vesicular stomatitis, etc.) 1.3. Minnesota, Pennsylvania, Kansas, Ohio, Missouri, and Georgia are conducting studies on hog cholera. Indiana, Iowa, Nebraska, and Michigan are working on atrophic rhinitis. Illinois, Nebraska, Indiana, and Michigan are working on transmissible gastroenteritis. Indiana and Georgia are working on erysipelas. The research on death losses in baby pigs was carried out under regional research project NC-13.

Industry and other organizations. Veterinary biological and pharmaceutical companies conduct research on the development and improvement of immunizing agents, drugs and antibiotics for the treatment and prevention of swine diseases. Preventive vaccines for hog cholera, erysipelas, and leptospirosis are among the products being investigated. Drugs and antibiotics for treatment of respiratory and enteric infections are being developed. Estimated annual expenditures are approximately 50 professional man years.

## REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Hog Cholera.

In 1961, hog cholera research at the National Animal Disease Laboratory, Ames, Iowa, was conducted in the following phases:

Occurrence of hog cholera after immunization. Investigations were made to determine the cause of cholera outbreaks in swine that had been vaccinated. Seventeen field specimens were studied. Three had no virus present and 14 had virus which were classified as follows: 3 variant viruses; 9 regular viruses, and 2 immunizing viruses. Seven of the regular virus were of such low virulence that all the pigs recovered after being sick 3 to 7 days. Salmonella and Pasteurella isolated from sick farm pigs caused cholera-susceptible and experimental pigs to become sick or die when infected simultaneously with modified hog cholera vaccines.

Propagation of hog cholera virus in vitro. Hog cholera hyperimmune serums prepared in pigs and rabbits, and serums from noninfected pigs and rabbits, were fractionated with Rivanol and ammonium sulfate. The gamma globulin fractions were conjugated with fluorescein isothiocyanate. Infected PK cells (a transmissible line of swine kidney cells), primary swine kidney cells, primary swine testicle cells, primary swine bone marrow cells, and noninfected cells of the same origins, were exposed to the labeled antibody. Smears prepared from the peripheral blood and lymph nodes of swine acutely sick with hog cholera, were treated the same as cultures. None of the infected cultures nor the smears were suitable for demonstration of specific immunofluorescence since the nonspecific reactions were of the same amount and intensity in both the infected and noninfected cells.



Studies were conducted on a PK swine kidney cell line persistently infected with an agent that immunized against hog cholera virus. The transmissible line of swine kidney cells (PK) was received from a commercial company. Apparent rapid modification of virulent virus used as inoculum and marked fluorescence of noninfected cultures suggested that the PK cells were already carriers of an agent antigenically related to hog cholera virus. The presence of the contaminating modified agent was demonstrated by immunizing susceptible pigs against hog cholera with supernatant fluid from an uninoculated PK cell culture. Confirmation that the cell strain was contaminated prior to its arrival at the Hog Cholera Station was demonstrated by immunizing susceptible pigs with another PK cell culture from the original source, immediately after receipt at the Station. It was not possible to eliminate this immunizing agent from the cell strain by using hog cholera hyperimmune serum in the tissue culture medium.

Immunizing properties of hog cholera vaccines. Investigations were made of the factors influencing the potency and immunizing ability of hog cholera vaccines in individual pigs and groups of pigs. One lot of virus (1840 ml) was made and tested for variant characteristics, but none were found. It had a titration of 2,500,000 minimum lethal doses per milliliter. This virus will be used for exposure of swine in various kinds of experiments and supplying experiment stations doing research on hog cholera.

An experiment was carried out to show the relative susceptibility to hog cholera virus of inbred lines of Hampshire, Duroc, Poland China, Yorkshire Landrace, and Chester-white pigs. Four of 6 Hampshires died; 5 of 6 Durocs died; 1 of 6 Poland-Chinas died; 1 of 5 Yorkshires died; none of the 6 Landrace died, and 1 of 2 Chester-whites died. The Durocs and Hampshires seem to be the most susceptible.

Crystal violet glycerol (CVG) vaccine, Sn. 116, that was made in 1959 and used to vaccinate farm herds in 1960, was re-tested and found to give 78 percent protection. The percent protection had not changed since being tested a year previous. Another vaccine, Sn. 117, consisting of 124,000 ml. was prepared in 5 sub-lots. Some of the sub-lots and the final pool were tested in Florida. All protection tests proved satisfactory. Part of this vaccine, 112,500 ml. was shipped to the Live Oak Station to be used in an experimental eradication trial in Lowndes County, Georgia.

CVG vaccination of 123 herds on 60 farms, composed of 12,088 pigs, was carried out in 1960-61. The immunity of a total of 486 pigs was challenged, 244 head at 1 month, and 242 head at 3 months after vaccination. The total percent protection for the year was 69.22. Last year it was 64.7. Death loss was 13.99 percent or 1.87 percent more than last year, although there was an increase of 13.13 percent in the number of normal pigs and a decrease of 7.78 percent and 7.55 percent in the slight and severe reactors, respectively. Two lots of vaccine serials 115 and 116 were used in this year's vaccination. Two viruses, serials 313 and 316, were used for the challenge of immunity.

Those herds which had little or no protection from single vaccination had 100 percent protection when given 2 doses of vaccine 1 month apart. Twenty-five sows, given 2 doses of CVG vaccine 1 month apart, had no reaction to exposure with virulent virus at 12 or 18 months after the last dose of vaccine.

The work on the reactivation of the viruses in modified vaccines was completed and published. Six modified viruses passed serially through pigs, all regained virulence so that they either made the pigs sick or caused death. Three of these viruses usually were transmitted from the injected pigs to the contact control pigs. The transmissibility by contact of the other 3 vaccines was not tested.

Some viruses of lapine origin regained their virulence in 6 passages. Other viruses of lapine origin required 19 passages before they produced sickness in susceptible pigs. A virus of porcine origin caused a severe reaction in pigs on the first passage when no serum was given with the virus. It increased in virulence on subsequent passages.

The addition of 0.15 percent of B-Propiolactone to hog blood that contained cholera virus and incubated at room temperature for 1 hour, killed the virus so that no reaction was produced when injected into susceptible pigs. These pigs developed sufficient immunity to survive a 2 ml. dose of virulent hog cholera virus. However, they were sick from 2 to 5 days. Similar results were obtained by adding 0.16 percent B-Propiolactone to blood containing hog cholera virus and incubating at room temperature for 30 minutes, or by adding 0.32 percent B-Propiolactone to blood containing hog cholera virus and incubating for 30 minutes.

Six accession lots of pigs had varying degrees of protection when 6 pigs from each lot were injected with graded doses of the same serial of vaccine and challenged with the same virus. Some lots had 100 percent protection while other lots had little or no protection.

Another lot of 42 pigs, composed of 6 breeds of pigs, seven in each breed, were all given graded doses of the same vaccine to determine if a genetic factor had any influence on their inability to develop immunity. All of the Poland China pigs died when challenged 21 days after vaccination. All except one of the Landrace died. All of the Chester-Whites survived. Four of the 6 Hampshires survived. Four of the Yorkshires survived, and 4 of the Durocs survived. This seems to indicate that there is a difference in breeds as to their ability to develop immunity.

In 1962, at the National Animal Disease Laboratory, immunizing studies were continued with the vaccination of a farm herd of 450 pigs, composed of three age groups, with crystal violet glycerol vaccine. One month later part of each group was given a second injection of CVG. Six months later representatives from each age group with single and double vaccinations were given hog cholera virus. Older pigs, vaccinated after weaning, were better protected than younger age groups. Double vaccination increased the protection 59 percent in the older group and 29 percent in the younger group. Double vaccination shortened the recovery period from 10 days to 8 days.



White cell counts of these animals showed that the rate and extent of drop in numbers of cells following virus challenge was about the same for single and double vaccinates of all age groups. The return to normal cell count was two days faster in the double vaccinates and the increase in number of cells was greater in the double vaccinates. The rate of recovery to a normal condition is related to the increase in leucocytes. Bacteriological studies of the hogs that died showed no pathogenic organisms present.

One lot of Crystal Violet Glycerol vaccine, consisting of 322 liters was made in 8 sublots. It is being tested for potency.

A total of 606 agar-gel precipitin diagnostic tests for hog cholera were made. Of 414 positive tests, 79 percent were obtained with normal pancreas material and 58 percent were obtained with pancreas from hog cholera infected animals.

The Taylor test for hog cholera, when made on a selected group of 22 virus-only-treated pigs and on vaccinated-and-virus treated hogs gave clear positive tests in 65.5 percent of the animals. The test was doubtful in 31 percent of the cases and 3.4 percent negative.

Bacteriological study of the organs from 630 hog cholera-infected pigs gave isolations of organisms from 114, divided in the following proportions: Escherichia coli 34; Staphylococcus spp. 29; Pseudomonas spp. 14; Streptococcus spp. 13; Proteus spp. 12; Diplococcus spp. 5; Aerobacter spp. 3; Corynebacterium spp. 2; Pasteurella spp. 1, and Escherichia fruendii 1.

Field evaluation of modified live-virus hog cholera vaccines. In 1961, 30,872 pigs from 1,023 herds on 517 farms in Suwannee County, Florida, were vaccinated with modified live-virus vaccines and antiserum. Approximately 85 percent of the swine in the county were vaccinated. Lapine origin vaccine was used on 35 percent of the pigs; porcine origin on 38.4 percent of the pigs, and tissue culture origin vaccine on 26.6 percent of the pigs.

Immunity tests on 661 randomly selected market-age swine from 340 herds showed that 82.5 percent of the pigs were adequately protected. This was an increase over the 78.1 percent protection from the previous year. The increase probably resulted from the use of fresh vaccines in recent months, and it supports the hypothesis that there is an inverse relationship between the age of the vaccine and its protective potency.

There were 13 confirmed hog cholera virus isolations; 7 from non-vaccinated swine and 6 from vaccinated swine. Thus more than 50 percent of the virus isolations came from the 15 percent of the swine that were not vaccinated. All 13 positive cases occurred during the first half of fiscal year 1961. This was a significant decrease from the 24 isolations in 1960. It appears that the high level of vaccination coverage has kept the disease well under control.

An investigation of immunization failure is centered around possible genetic resistance to immunization. Breeding stock was obtained from a herd from which only 10 of 19 animals were adequately protected by vaccination. In



preliminary studies, 2 of 6 first-generation pigs were susceptible to virus challenge given 104 days after vaccination with modified live-virus vaccines. One of 2 second-generation pigs was susceptible when given an immunity challenge 80 days after vaccination.

Titration of 6 different vaccines were carried out to determine minimum protective doses. There was a great variation of potencies among the vaccines tested. Further work is needed on the titration of vaccines as soon as possible after production and at 6-month intervals until the expiration date in order to plot a reliable immunogenicity retrogression curve.

In 1962 in Suwannee County, Florida, 9,307 swine were vaccinated against hog cholera with lapine origin vaccine, 295 of them were challenged, and 260, or 88.1 percent were adequately protected. A total of 10,931 swine were vaccinated with porcine origin vaccine, 321 of them were challenged and 278, or 86.6 percent were adequately protected. A total of 9,622 swine were vaccinated with tissue culture vaccine, 191 of them were challenged and 165, or 86.4 percent were adequately protected. The totals for all types combined were 29,860 swine vaccinated, 807 challenged, and 703, or 87.1 percent adequately protected.

Porcine origin vaccines recovered from a low percentage of adequately protected pigs of 61.6 percent in fiscal year 1960 to a high of 86.6 percent in fiscal year 1962. The average age of porcine origin vaccines (shelf-life) in fiscal year 1960 was 564.4 days (more than 18 months) whereas the age of the same type of vaccine in fiscal year 1962 was only 206.3 days (less than 7 months). The poor showing of porcine origin vaccine in fiscal year 1960 involved 8 serial numbers from 4 manufacturers. The recovery of this vaccine in fiscal year 1962 involved 6 serial numbers from 3 manufacturers.

The efficacy of all porcine origin vaccines during the past 6 years shows approximately the same pattern as described above. The average percentage of adequately protected pigs was 88.5 when the vaccines were less than 1 year old. When the vaccines were between 1 year and 18 months of age, the percentage of adequately protected pigs dropped to 80.3, and when the vaccines were more than 18 months of age, this figure was 57.9 percent. Lapine origin vaccines and tissue culture vaccines showed similar correlative declines but they were not as marked and not as early. With lapine origin vaccines, 88.5 percent of pigs were adequately protected if the vaccines were less than 18 months of age, whereas, this figure was only 80.9 percent when the vaccines were more than 18 months of age. (The minimum acceptable percentage of adequately protected pigs is 80.0 percent). With tissue culture vaccines, 91.9 percent of pigs were adequately protected when the vaccines were less than 18 months of age, and 87.8 percent were adequately protected when the vaccines were more than 18 months of age.

During fiscal year 1962, a field trial study was started in Lowndes County, Georgia, in cooperation with the Animal Disease Eradication Division of ARS and the State of Georgia, under the terms of a Memorandum of Understanding. This arrangement was similar to the one in Suwannee County, Florida, to determine the efficacy of inactivated and killed vaccines. Formal agreements were entered into with 703 swine owners, which is about 97 percent of the total swine owners in the county.



During fiscal year 1962, in Lowndes County, Georgia, a total of 23,899 swine were vaccinated with Boynton's Tissue Vaccine (BTV), and experimental Crystal Violet vaccine (ECVG) and a commercial CVG vaccine (CCVG). A total of 5,289 swine were vaccinated against hog cholera with BTV, 9 of them were challenged and all 9 were adequately protected. A total of 9,963 swine were vaccinated with ECVG vaccine, 50 of them were challenged and 49 were adequately protected. A total of 8,647 swine were vaccinated with CCVG vaccine, 10 of them were challenged and all 10 were adequately protected. Additional swine receiving each type of vaccine will be challenged when they reach market age.

Three positive cases of hog cholera in Suwannee County, Florida, were disclosed in non-vaccinated, farm-raised swine on 2 farms and in vaccinated, farm-raised swine on 1 farm. In Lowndes County, Georgia, hog cholera was confirmed in purchased, non-vaccinated swine on 1 farm.

In other tests, in Suwannee County, Florida, it was shown that 2 doses of inactivated vaccine administered 30 days apart, imparts almost 100 percent immunity, even if serum is administered simultaneously with the first dose.

Development of a rapid diagnostic test for hog cholera. In January 1962, this work, carried out at the University of Illinois, under a cooperative agreement was initiated. Preliminary investigation indicates that a hem-agglutination test has promise as a rapid diagnostic test for hog cholera.

The relationship of hog cholera to bovine mucosal disease. In 1962 work was carried out under a cooperative agreement on the mucosal disease-virus diarrhea complex of cattle at the College of Veterinary Medicine, Iowa State University. The experimental results indicate that the Sanders mucosal disease agent does not give uniform protection to swine against virulent hog cholera virus. The results definitely indicate that the Sanders Mucosal Disease Agents affords protection in some of the swine against virulent hog cholera virus.

One experiment, consisting of 8 pigs, has been conducted in this area of study so far. The pigs were divided into 4 lots of 2 each. Two pigs were inoculated intravenously; 2 subcutaneously and 2 intramuscularly with Sanders Mucosal Disease Agent. Two pigs remained as controls. The only evidence of response to the inoculation was a drop of about 50 percent in the total leukocytes between the 4th and 6th days postinoculation.

Nineteen days later all 8 pigs were inoculated subcutaneously with virulent hog cholera virus. The 2 control pigs developed clinical evidence of hog cholera 3 days postinoculation and were destroyed in extremis on the 9th and 11th days postinoculation. Typical lesions of hog cholera were observed at necropsy.

One of the pigs which had been inoculated intravenously with Sanders agent developed clinical symptoms of hog cholera on the 2nd day post-challenge and was destroyed in extremis 15 days later. Lesions of hog cholera were evident at necropsy. The second pig to receive the intravenous inoculation of Sanders Agent displayed a mild pyrexia, depression, and weakness in the rear quarters between the 4th and 14th days post-challenge. After this period the pig showed no evidence of illness and apparently returned to normal health.



Of the two pigs that received the Sanders Agent intramuscularly, one showed little evidence of infection following the hog cholera challenge, the other pig, however, was quite ill between the 4th and 12th days post-challenge. During this period the temperature varied from normal to 106°F. The animal was very weak, depressed and ate very sparingly. After this period the pig rapidly returned to normal.

One of the two pigs that received the subcutaneous inoculation of Sanders Agent developed typical symptoms of hog cholera on the 3rd day post-challenge and was destroyed in extremis on the 9th day post-challenge. Lesions of hog cholera were evident at necropsy. The other pig to receive the Sanders inoculation subcutaneously developed symptoms 6 days post-challenge and was ill for 7 days. During this time the pig was markedly depressed, off feed and showed a variable temperature elevation. Following this period the pig rapidly returned to normal.

#### B. Atrophic Rhinitis.

In 1961, at the Animal Disease Station, Beltsville, Maryland, studies were carried out on the causative agent or agents, mode of spread, diagnosis, and control of atrophic rhinitis. Progress in the atrophic rhinitis (AR) project is developing methods of inoculation and storage of inoculum pools during this period was satisfactory, and data obtained further substantiated findings of previous years. The contributions were (1) frozen inoculum will remain viable for at least 8 months, whereas previous tests of frozen inoculum showed viability for only 4 months; (2) the material can be diluted out at least 40 times and still remain infective. Previous tests were made only with dilutions of 1:3.5; (3) the transmissible nature of AR infected nasal turbinate mucus membrane tissue was clarified by experiments testing the noninfected materials and conditions associated with positive transmission. Air alone, saline alone, or normal nasal turbinate mucus membrane tissue did not produce atrophy of the turbinates when instilled into the nasal cavity of susceptible pigs by means of the DeVilbiss atomizer with an electric pump at 13 pounds pressure; (4) the atrophic rhinitis susceptibility of pigs 14 to 25 days of age was similar to previous years experimental results with pigs under 14 days of age, and a few swine over 2 months of age, when tested, were susceptible to atrophic rhinitis exposure; (5) streptomycin or a combination including streptomycin, will inhibit atrophy-producing ability when mixed with the inoculum, whereas other antibiotics tested (penicillin, polymyxin or bacitracin) will not; (6) data obtained shows lyophilization seems to be the poorest method of storage of the inoculum; (7) the use of the rhinoscope may be of some value for diagnosing AR in a herd, but is not efficient enough for the critical work required of it for research.

In 1962 work was initiated at the National Animal Disease Laboratory, Ames, Iowa. Work during the past year was associated with the hiring and training of new project personnel, testing the established AR-free swine herd for susceptibility to the disease, developing and testing new animal cage isolation equipment and testing the deep freeze stored AR material from Beltsville for viability and transmissibility to susceptible pigs. The development of closed air and sewage systems in a plexiglass Horsfal-Bauer type isolation cage, 24"x24"x36" used in 3 experiments maintained satisfactory isolation between AR infected and normal control pigs up to 100 pounds live weight for approximately 60 days. The 20 AR-free, purebred Yorkshire sows moved from Beltsville, Maryland, have provided a satisfactory source of susceptible pigs. The atrophic rhinitis frozen agent harvested and shipped from Beltsville was viable and transmissible to pigs in a 1:3.5 dilution after 16 months storage. It was transmissible to pigs in a 1:56 titration dilution after 18½ months storage. However, the pig passage of epithelial tissue harvested from the NADL experimental pigs was negative. Swine age susceptibility tests are in progress.



### C. Transmissible Gastroenteritis

In 1961 preliminary work at the National Animal Disease Laboratory, Ames, Iowa, was directed toward producing disease-free swine for use in controlled experiments on transmissible gastroenteritis (TGE).

In 1962 at NADL, two lines of cells, one from fetal swine testis and one from fetal swine kidney, were developed in this laboratory for use in research on TGE. Only the swine testis cells have been used thus far. Characteristically these cells grow very slowly and require 5 percent CO<sub>2</sub> in the atmosphere for optimum growth. The cells have been passed only 16 passages in the 8 months since they were started. Eight passages were made in the first 3 months and 8 passages have been made in the last 5 months.

Investigations utilizing two isolates of transmissible gastroenteritis have been undertaken. One isolate obtained from a natural field outbreak in Central Iowa, and a second isolate received from the Purdue University, were used. It is the original Indiana isolate of TGE. Both isolates were pathogenic for very young specific-pathogen-free (SPF) pigs.

The Iowa isolate was inoculated into the swine testis cells at the 8th passage of the cells. The cytoplasm of many of the cells became granular and there was a marked acceleration growth pattern of the passaged cells as compared with noninoculated control cell line. The persistently infected cells have been passed 20 times since they were infected 5 months previously. They grow well without CO<sub>2</sub> atmosphere and in Earle's balanced salt solution and 10 percent pig serum.

Positive evidence of viral invasion of the cells was obtained by feeding 2 six-day-old SPF pigs infected cells after they had been passed 15 times. The infected pigs showed no overt signs of disease, but when they were challenged, along with the control pig, with the original homologous virus isolate, only the control pig developed clinical evidence of TGE.

The Purdue isolate was passed 3 times in an established line of pig kidney cells obtained from another laboratory. There was no evidence of infection or multiplication of the virus in these tissue culture cells. Positive evidence of viral invasion of the cells was indicated when the 3rd passage of the tissue culture passed virus was fed to each of two 6-day-old SPF pigs. The virus appeared to be partially attenuated as only one pig developed signs of TGE and died. This pig had lesions suggestive of TGE. The second pig showed no signs of disease. When the remaining pig and a control pig were challenged with pig passaged homologous virus only the control pig developed clinical evidence of TGE.

In 1961, under a cooperative agreement with the University of California, at Davis, studies were conducted with 4 of 6 viral agents isolated from the intestine of young pigs suffering from an acute scouring in the field. Investigations were conducted to determine the optimum conditions under which the 4 agents could be propagated and demonstrated by tissue culture methods.

A neutralization test, using the tissue culture system, showed an antigenic relationship among the viruses obtained from pigs with scours which were serologically unrelated to a viral agent obtained from an apparently normal hog.

Investigations on the physical properties of the viruses were carried out. Exposure at 56 C for 40 minutes inactivated 90 percent of the virus but infectious material was still present after 2 hours; after 5 hours no infectious virus remained. The viruses were resistant to 20 percent ether, 1 percent trypsin, and were viable after 300 days at -20 C. None of the viruses hemagglutinated red blood cells of chickens, guinea pigs, sheep, or swine.

Preliminary trials with one of the agents obtained from the scouring pig resulted in signs of leg weakness, paralysis and definite histopathological lesions in the brain stem and spinal cord, when inoculated into 24-hour-old specific pathogen-free pigs.

In 1962, at the University of California, physical, serological and pathological studies were performed with four strains of porcine enteroviruses designated as E1, E2, E3, and E4. The particle size of these agents was estimated to be 33 to 46 mu in diameter by gradocol membrane filtration. The relationship between these four strains was studied by agar gel diffusion test, and further work is now in progress.

The porcine enterovirus (PE-1 strain) isolated by the Canadians differed from our four strains as determined by the tissue culture plaque neutralization test. Pathogenesis of the four strains was studied by oral inoculation into SPF pigs. In most cases, the pigs inoculated with E1, E2, or E3 strain showed signs of gastro-intestinal enteritis, and the virus was isolated from the lower intestinal tract. One of five pigs inoculated orally with the E1 strain showed paralysis in the hind legs and poliomyelitis lesions were demonstrated in the spinal cord by histopathological examination.

In 1961, under a cooperative agreement with Purdue University, Lafayette, Indiana, duration of immunity to transmissible gastroenteritis was tested by immunizing 6 gilts by oral exposure to virulent virus during pregnancy. In the first farrowing their pigs had morbidity of 19 percent and mortality of 12 percent. Fifteen months after the first farrowing, the sows farrowed again and on challenge of the pigs at 3 days of age, the pigs showed morbidity of 11 percent and mortality of 8 percent. Three of 4 sows conferred solid immunity to their pigs. To test the duration of the carrier state, fecal samples were taken from 35 pigs at weekly intervals for 11 weeks and tested for the presence of TGE virus. The 35 pigs were left with the sows until weaned at 6 to 7 weeks. Of 375 fecal samples, 188 were tested by inoculating individual pigs in isolation. TGE virus was demonstrated in only 10 of 35 pigs in the first week, from 2 pigs in the second week, and none in the weeks thereafter. The results are at variance with the only previous report on the subject in which pigs shed TGE virus for as long as 8 weeks after exposure.



In 1962, at Purdue University, two phases of immunity to TGE were studied. In continuing the work on duration of immunity, it was found that gilts infected as suckling pigs between 17 and 25 days of age did not retain sufficient immunity to protect their litters from our standard challenge after one year. There was some prolongation of incubation period and some diminution in death loss over controls.

Antibodies absorbed from colostrum through the gut of the newborn animal are considered to be the important mechanism of transfer of immunity from dam to young in most farm species including swine. Experiments were done in which pigs were transferred from immune sows to non-immune sows and vice versa. It was shown that immunity against TGE transferred from sow to pig is dependent upon a continuous supply of "immune" milk. Intraperitoneally inoculated anti-serum failed to protect pigs while the same anti-serum protected when fed in the milk indicating that the important site of action is probably in the lumen or walls of the alimentary tract.

Persistence of virus shedding was not studied extensively because this work requires large numbers of pigs which were in temporarily short supply. However, it was found again that three infected pigs did not shed virus after one week. Four-day-old pigs shed as much as 10,000 infectious doses of virus per gram of feces as early as 24 hours after infection.

New approaches to adapt TGE virus to tissue cultures were made this year involving kidney monolayer cultures, organotypic cultures of various visceral organs including various parts of the gut, and a modified organ culture technique patterned after Warren's technique for growing polio-virus in monkey gut. While none of these was completely successful, the method of growing explants of intestine on millipore filters is being developed and in the best cultures would at least maintain TGE virus for three to four days.

#### D. Swine Erysipelas

In 1961, at the Animal Disease Station, Beltsville, Maryland, fifty pigs, farrowed by vaccinated swine-erysipelas-immune dams, were divided into 5 groups of 10 pigs each after weaning. They were vaccinated with anti-swine erysipelas serum Lot G, and commercial desiccated virulent Ery. rhusiopathiae at different mean ages after birth (59.7 to 115.7 days). All were exposed to infection 94 days after vaccination, including 14 pen-contact controls, by the percutaneous method of exposure. There was a trend of improvement in the immunizing response to vaccination as the age when vaccinated was increased. All pen contacts were susceptible. The experimental results indicated that pigs farrowed by immune dams should not be vaccinated with virulent culture and serum until after they are 3 months old.

Biochemical investigations were carried out in cooperation with the Department of Biochemistry, Seton Hall College of Medicine and Dentistry, Jersey City, New Jersey. Buffer extracts of Ery. rhusiopathiae were resolved into 5 antigens that were distinguishable on the basis of (1) chromatographic properties, (2) heat stability, and (3) reaction of non-identity in agar diffusion studies. The antigens were identified as homologous, isologous and heterologous with respect to known serotypes.

In 1962, at the National Animal Disease Laboratory, Ames, Iowa, work was carried out as follows: 1) No gross evidence of arthritis was observed in rabbits after sensitization to Erysipelothrix rhusiopathiae in combination with Freund's adjuvant, when injected intradermally in the interdigital space and side. 2) Acetone powder was prepared from 147 liters of broth culture for the immunological and chemical characterization of the antigen components of Ery. rhusiopathiae. 3) Peak A, the type-specific antigen derived from acetone powder, is identical with the acid extracts of the cells that formed the basis for the original classification of strains of E. rhusiopathiae.

Biochemical investigations were continued cooperatively with the Department of Biochemistry, Seton Hall College of Medicine and Dentistry, Jersey City, New Jersey. Ion-exchange chromatography of neutral extracts of acetone powders of Ery. rhusiopathiae strain S-192 on DEAE-cellulose columns resulted in the isolation of 5 serologically distinct antigens when assayed by the agar double diffusion technique. Peak A, the "breakthrough" peak from the column precipitated only with homologous anti S-192 serum and the antiserum to a closely related strain NF<sup>4</sup>.

During the period covered by this report, purification of the serologically active material in Peak A by chromatography, acetone fractionation, and exhaustive dialysis yielded a preparation which had all of the serological activity of the original Peak A.

Studies on the chemical composition of this type-specific antigen have indicated that it is a mucopeptide most probably derived from the cell wall of the organism. It has been demonstrated that the antigen isolated in this manner from acetone powders of the organism is identical both chemically and serologically with the antigen present in acid extracts of the organism. It was acid extracts of cells of Erysipelothrix which were used originally to type the various strains of the organism.

#### E. Brucellosis.

Under a cooperative agreement on bovine brucellosis, reported in Area 1, with the College of Veterinary Medicine of the University of Minnesota, studies on brucellosis in swine were undertaken in April of 1962.

Sows and boars which react to the tube seroagglutination test for brucellosis at the time of slaughter are being traced to the farm of origin. These selected herds are followed and evaluated using epidemiologic and serologic methods developed for the detection of frank and inapparent infections of brucellosis in cattle. These studies include attempts to isolate the organism from all animals which leave the herd for slaughter using culture media, guinea pig inoculation, and fluorescent antibody microscopy.

Blood has been collected from 363 sows which have been identified prior to slaughter in order that all of the animals from which blood was collected may be traced to the herd of origin. These sows are selected with the cooperation of a packing plant, and the herds of origin are located in southern



Minnesota and northern Iowa. Initial serological studies include the plate sero-agglutination test, tube sero-agglutination test, acid plate antigen test, heat inactivation test, rivanol precipitation plate sero-agglutination test and in some cases the 56°F heat inactivation test. In the herd studies, the 56° HIT and MCE tests will be included on all animals. Preliminary results indicate that the number of reactors to the plate and tube agglutination test at dilutions of 1:100 and greater are quite low; however, approximately 10% of the animals show plate or tube reactions at 1:25 or 1:50 dilutions. The results of the supplemental tests are now being evaluated. Trace-backs are in progress to locate the herds of origin of those animals which reacted.

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FOOT-AND-MOUTH DISEASE AND OTHER  
EXOTIC DISEASES OF SWINE  
Animal Disease and Parasite Research Division, ARS

Problem. Foreign diseases such as foot-and-mouth disease, African swine fever, and Teschen disease, that occur elsewhere in the world, constitute calculable potential threats to the swine industry of the United States. Foot-and-mouth disease is of particular importance because the disease frequently occurs primarily in swine from which it spreads to other susceptible species, such as cattle and other ruminants. African swine fever, which until recently was confined to wild and domestic pigs in Africa, has spread to Portugal and Spain. The disease is of special concern because of its resemblance to hog cholera, with which it may be confused. Moreover, mortality from the disease approaches 100 per cent, and there is no specific preventive vaccine. Teschen disease, which causes widespread inapparent infections and occasional involvement of the central nervous system, is another of the foreign diseases to be guarded against. A disease indistinguishable from Teschen disease has appeared in England in recent years. Despite all precautions, any of these diseases may occur in the United States, as likely as not through the medium of modern, rapid international transportation. The Plum Island Animal Disease Laboratory is engaged in studies of foreign diseases of swine, for the purpose of developing information for increased protection of the Nation's swine industry.

USDA PROGRAM

The Department has a continuing long-term program involving veterinarians, biochemists, microbiologists, and pathologists, engaged in basic and applied research in this problem area. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 5.6 professional man years. This effort is divided among sub-headings as follows:

Foot-and-Mouth Disease of Swine 1.0 at the Plum Island Animal Disease Laboratory, Plum Island, New York.

African Swine Fever 4.6 at the Plum Island Animal Disease Laboratory in cooperation with the East African Veterinary Research Organization, Muguga, Kenya, and in connection with a P.L. 480 project in Madrid, Spain, where the equivalent of \$97,550 has been made available to the Spanish Ministry of Agriculture over a 3-year period.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

Owing to prohibition of experimentation with these diseases in the United States, except at the Plum Island Laboratory, no work in this area is being done in this country at State Experiment Stations or by American industry.



## REPORT OF PROGRESS FOR USDA

A. Foot-and-mouth Disease

During 1961 at the Plum Island Animal Disease Laboratory, immunological investigations of foot-and-mouth disease of swine were carried out. The response of a group of swine to infection with foot-and-mouth disease virus was determined. These studies confirmed the concept that fundamental differences exist in immune response between swine and cattle. Swine respond to infection with rapid appearance of antibody which drops to low levels within four months, permitting some of the animals to become reinfected by contact with infected animals. Vaccination of swine with formalized tissue culture vaccine has generally been unsatisfactory.

During 1962 research effort has been directed toward the development of assay procedures suitable for study of antibody in swine serum. Experimentation has been directed toward determination of the antibody response after infection. Pretreatment of swine serum with dilute solutions of formaldehyde completely removed the procomplement activity and permitted detection of FMD antibody by complement-fixing methods. This agreed with previous findings in work with African swine fever virus. In some trials, however, it has been observed that complete fixation did not occur unless normal bovine serum was included in the mixture.

B. African Swine Fever

In 1961 investigations were continued in Kenya in cooperation with the staff of the East African Veterinary Research Organization. Bush pigs, as well as wart hogs, have been found to be inapparent carriers of the disease. Three virus isolations were made from 76 bush pig specimens examined.

Since African swine fever has become widespread in Spain and Portugal there is even greater need for methods of differentiating ASF from hog cholera, and for a better understanding of the mechanism of infection with ASF virus and other aspects of the disease that are important in prevention, control, and eradication.

An improved and simplified method of preparing pig leukocyte cultures has made possible production of uniform cultures for routine use in assaying ASFV. These cultures are being used to measure rates of virus inactivation occurring at various levels of pH and temperature.

By alternate passages in pig leukocyte and chicken embryo cell cultures it has been possible to adapt several strains of ASF virus first to chicken embryo cells and then to a line of pig kidney cells.

The enhancement of the complement-fixation reaction by the addition of normal bovine serum has made possible the detection of complement-fixation antigen in extracts of spleen and lymph nodes from pigs infected with ASFV. Additional work is required to establish the reliability of the technique.

In preliminary studies, certain of the antigenic components, active in the agar-gel precipitin test, are destroyed by proteolytic enzymes while others are not. The antigens also vary in their heat stability.

In 1962 epizootiological studies have continued to determine the incidence of carriers of the virus among wild species of animals in Kenya. In addition, work is being conducted to determine the number of types of virus in order that a logical approach may be made to develop immunizing agents.

Work has continued to adapt various strains of ASF virus to cell cultures. Methods for adapting ASFV to cell cultures have been established and several isolates have been serially propagated in chick embryo cells and a line of pig kidney cells. There has been evidence of some modification of the virus following passage in cell cultures. More isolates will be adapted and serially propagated in cell cultures to obtain one or more attenuated strains which may serve as immunizing agents against all strains of ASFV.

Work is also continuing to develop means of propagation of quantities of ASFV by cell culture methods. Quantities of the virus will be especially useful in studies on virus inactivation and production of viral antigens. Serological investigations have continued and it has been shown that swine which survive the acute stage of ASF develop complement-fixing antibodies against antigens in infected tissue culture. The development of a means of serological diagnosis is a significant advancement and one which should supplement diagnosis by the hemadsorption test which was developed several years ago. Diagnosis, however, by serological methods would permit a more rapid means of distinguishing ASF from hog cholera than may be done with the present hemadsorption technique.

The epizootiological studies have shown that bush pigs, wart hogs, and porcupines may be inapparent carriers of ASFV.

The work under the P.L. 480 project in Spain is primarily on diagnostic studies. The hemadsorption test was applied to specimens from 243 suspected outbreaks of ASF. Of these, 172 were positive, 67 were negative, and 4 were toxic to the leukocyte cultures. In these investigations the hemadsorption effect was specific for African swine fever and bacterial contamination did not interfere with the test.



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PARASITES AND PARASITIC DISEASES OF SWINE  
Animal Disease and Parasite Research Division, ARS

Problem. Parasitic diseases have been estimated to cost the swine industry of the United States at least \$200 million annually. These diseases for the most part are cosmopolitan. Subclinical infections are the most frequent type and the most costly, yet they are generally so difficult to recognize that they often are overlooked entirely. Diagnosis is difficult, and successful treatments for many of these parasitisms are not available. Moreover, management practices to avoid the spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling, or eradicating parasitic diseases so as to provide for healthy swine, insure adequate supplies of parasite-free pork for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a prosperous agriculture, a sound national economy, a high standard of living, and a healthy population.

USDA PROGRAM

The Department has a continuing long-term program involving parasitologists, veterinarians, biochemists, microbiologists, and pathologists engaged in basic and applied research in this problem area. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 4.7 professional man years. This effort is divided among sub-headings as follows:

The role of parasites in the economy of swine production 1.2 at the Beltsville Parasitological Laboratory, Beltsville, Maryland, and at the Division's laboratory at Tifton, Georgia, through informal cooperation with the Georgia Coastal Plain Experiment Station.

Bionomics and pathogenicity of the swine whipworm 0.5 at the Beltsville Parasitological Laboratory.

Swine kidney worms 2.1 at Tifton, Georgia, the Beltsville Parasitological Laboratory, and under cooperative agreement with the North Carolina Agricultural Experiment Station at Raleigh.

Investigations of *Trichinella spiralis* 0.5 at the Beltsville Parasitological Laboratory.

Effect of anthelmintic treatment on rate of gain 0.3 at Tifton, Georgia.

Pathogenic role of the intestinal roundworm 0.1 under a cooperative agreement with the Nebraska Agricultural Experiment Station at Lincoln.



## RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 3.9 professional man years divided among sub-headings as follows: effect of anthelmintic treatment on rate of gain 0.4; swine kidney worms 0.5; pathogenic role of the intestinal roundworm 1.0; biology and pathology of the swine whipworm and other parasites 2.0. Work in the North Central Region deals with anthelmintic treatment, roundworms, and biology and pathology of the swine whipworm and other parasites. Work in the Southern Region deals with anthelmintic treatment, roundworms, and kidney worms. The Western Region is carrying out work on anthelmintic treatment.

Industry and other organizations. Several chemical companies are engaged in the formulation of compounds and explorations for chemicals that may be used safely in the treatment of swine for elimination of worms. Generally, these companies have their own facilities, including laboratories, barns, and other structures containing pens for experimental animals, and in some cases, pastures. The work of these companies and the results, expenditures, and related matters are ordinarily confidential, since they involve eventually saleable products. Estimated annual expenditures are equivalent to approximately 15 professional man years.

## REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. The role of parasites in the economy of swine production.

In 1961, at the Beltsville Parasitological Laboratory, studies of the lipid content of helminth parasites were initiated in an attempt to learn more about the fat metabolism of helminths. This type of investigation was only made possible by the development of new techniques and information obtained was useful in increasing our understanding of parasitic disease and of developing new methods for the control of parasites by chemical and biological methods. Four species of helminths and one species of lice were analyzed in this preliminary study and several higher carbon-number fatty acids were found to be present. The lipids in the helminths (swine nodular worms) were similar to those found in the intestinal mucus in which they live. The presence of odd-carbon-number fatty acids in these parasites indicated that the worms may feed on bacteria. The lipids of the louse were somewhat different from those found in the blood of the host. This finding indicated that the lice either metabolize the lipids or concentrate them.

One of the nodular worms of swine, Oesophagostomum quadrispinulatum, was found to consist of possibly three different strains. Two of these have a 20-day prepatent period, whereas the other has one of 50 days. One of the 20-day strains has tails shorter than the other 20-day strain. The tails of the latter and of the 50-day strain are relatively long. There is some evidence that the short-tailed form can be used for genetic studies.

The search for cheaper and more satisfactory materials than animal charcoal for use in making cultures of round worm eggs in swine feces resulted in the finding that peat moss, sphagnum moss, vermiculite, sawdust, sand, and topsoil all produce satisfactory results. Peat moss was considered the most satisfactory material for culturing eggs of the nodular worms and red stomach worms of swine. The relative quantities of water, feces, and the above-named materials and the thoroughness with which the ingredients were mixed were found to be quite important in obtaining large yields of infective larvae. The quantity of the various ingredients vary with the amount of moisture in the fecal sample. However, satisfactory results were obtained when 40 to 80 ml. of water, 20 grams of peat moss to 595 grams of sand were mixed with 100 grams of fecal material.

Studies indicated that adult nodular worms can become established in another swine host after being transferred to it by means of an enema tube via the anus. Seven pigs were successfully infected by this means.

Infective larvae of swine nodular worms were observed to be capable of infecting susceptible pigs after being stored at ordinary refrigerator temperatures (4°C.) for 575 days.

In 1962 further investigation of the lipids in the tissues of the swine nodular worm, Oesophagostomum quadrispinulatum, demonstrated that all five kinds of phospholipids were present - non-choline phospholipids, inositol phosphatide, lecithin, sphingomyelin, and lysolecithin. In addition, studies on two intestinal nematodes of chickens, Ascaridia galli and Heterakis gallinarum, the pork muscle worm, Trichinella spiralis, and one free-living nematode, Ditylenchus myceliophagus, disclosed the presence of 28, 33, 30, and 28 fatty acids, respectively. All of the last-mentioned compounds were straight chain fatty acids, whereas 10 of the 33 fatty acids reported from the swine nodular worm in 1961 had side chains. The three parasitic nematodes had a complete series of saturated straight chain fatty acids ranging from 9 to 20 carbon atoms. Fatty acids having 9, 13, 17, and 19 carbon atoms were missing from the free-living nematode. From 44 to 58 percent of the fatty acids in the parasitic worms were unsaturated, whereas 65 percent of those in the free-living nematode were in this group. The fatty acid having 16 carbon atoms and two double bonds was prevalent in the nematodes from the chicken. The most abundant fatty acids in the free-living nematode were those with 18 and 22 carbon atoms having one unsaturated bond. The significance of these findings is yet to be determined.

In 1962 at Tifton, Georgia, the effect of Strongyloides ransomi on the rate of gain of pigs of different ages and on different levels of nutrition was less than the effect of the age of the pigs and the levels of nutrition as compared with inoculated and non-inoculated, full-fed and limited-fed, and 80 lb. and 40 lb. groups. This was attributed mainly to the late establishment of infections in the inoculated groups of pigs.



Strongyloides ransomi infections in baby pigs may cause death or serious loss in thriftiness. Diagnosis of natural infections and observations of experimental inoculations given to pregnant sows indicate the possibility of pre-natal infections of this species as a common occurrence.

Diagnosis of field cases of parasitism again showed Strongyloides ransomi to be the most prevalent parasite to cause death and unthriftiness in baby pigs. Ostertagia ostertagi was the most prevalent species in clinical cases of parasitism in cattle and was the cause of the loss of 6 brood cattle of 3 to 6 years of age.

#### B. Bionomics and pathogenicity of the swine whipworm.

In 1961, at the Beltsville Parasitological Laboratory, observations on the period during which swine whipworm eggs, placed on the surface or buried in soil up to 8 inches, remain infective to pigs in the vicinity of Beltsville were continued. Eggs deposited during the summer months survived 4 and 1/2 years on the surface of the plots, and at depths of 4 and 8 inches. Eggs deposited during the winter months have survived 2 years on the surface of the soil and at a depth of 4 inches. However, samples from the surface of plots contaminated in June 1956, did not produce infections in pigs when administered to them in April 1961. The infectivity of the buried eggs was not tested at that time. These results demonstrated that the swine whipworm egg remains infective to pigs for as long as 4 and 1/2 years in the absence of recontamination, whether on the surface of the soil or buried as deep as 8 inches.

Several attempts were made to find and describe the early stages of the parasitic portion of the life cycle of the swine whipworm, which up to the present time have not been described. Pigs were fed 50,000 embryonated eggs of Trichuris suis and were necropsied on the 7th, 9th, 13th, 21st, and 23rd day after infection. In stained sections of the caecum and large intestine, portions of nematode larvae were found deeply embedded in the crypts of the mucosa on the 9th day, and on the surface of the mucosa on the 13th day. A few first-stage larvae were recovered from the lumen of the large intestine and cecum on the 23rd day, and the first- and second-stage larvae on the 21st day.

#### C. Swine kidney worms.

In 1961, at Tifton, Georgia, the program for the eradication of kidney worms from an experimentally infected area at the Georgia Coastal Plains Experiment Station was completed. The program was based on limiting the breeding herd to young gilts and disposing of them after weaning their first litter. The incidence of kidney worm infections in pigs on contaminated lots was reduced from 88 percent (Spring, 1959) to 34 percent (Fall, 1959) to 0 percent in the Spring of 1960. A similar experiment is under way on a farm where a heavy natural infection of kidney worms was prevalent.

In 1962, at Tifton, Georgia, it was reported that the incidence of kidney worm infection in pigs on the experimental farm, raised from gilts in a kidney worm contaminated lot, was 93 percent for the spring group of 1961 pigs and 50 percent for the fall group. The incidence in pigs farrowed in an adjacent lot from gilts free of kidney worms was zero for both the spring and fall groups.

In 1961, in work performed under a cooperative agreement with the North Carolina Agricultural Experiment Station, Raleigh, there were indications that pre-natal infection with swine kidney worms might occur if sows were exposed to kidney worm larvae during pregnancy. In pigs fed kidney worm larvae at 8 weeks of age, eosinophilia appeared at the second week and reached a peak at the seventh to ninth weeks, and continued at a high level through the sixteenth week. The sedimentation rate increased at the fifth week and continued at a high rate through the sixteenth week. A series of compounds, including the organic phosphate group, failed to stop ova production in kidney worm-infected sows when administered orally or by inoculation within a safe level of medication.

In 1962, at the North Carolina Station, in studies to substantiate prenatal infection, six gilts reared colostrum-free in air-lock isolation units, were given 500 infective *S. dentatus* larvae weekly. These doses began at first heat and will continue throughout gestation. The gilts were bred at second heat period. Arrangements have been made to obtain colostrum- and parasite-free pigs to be penned with the offspring of these gilts for positive controls. The pigs from the gilts will be weaned at 8 weeks of age and the sows autopsied to determine status of kidney worm infection in the dams. Blood samples will be taken from the pigs for cytological and immunophoretic studies. Postmortem examinations will be made of pigs from different litters at 6 and 12 months of age.

Sows were purchased from a local abattoir following demonstration of ova in urine. They were confined in a quonset hut with concrete floors that were scrubbed daily to remove source of reinfection. The ova in the urine of these sows were used as a source of infection for experimental animals. A large Duroc sow is passing viable ova in urine after three years. There was a slight drop in concentration of ova after 30 months. A Poland China sow is producing large numbers of ova after confinement for 16 months.

Several compounds have been evaluated as possible anthelmintics against the swine kidney worm. Naturally infected sows were purchased following the demonstration of ova in the urine. The compound under investigation was given orally or as an injection. Examinations were made of the urine to determine influence on ova production. Compounds evaluated included Bayer 13/59, Bayer 21/199, Bayer 29493, Ruelene, and Tenneccetin. No satisfactory compound was found that decreased ova production within a safe level of medication.

Substantiation of prenatal infection would aid in the understanding of the life cycle of the kidney worm. This phenomenon could shorten the prepatent period by 2 to 3 months and help explain the presence of sexually mature parasites in young pigs. A patent infection in sows for as long as 3 years would greatly intensify the problem of control, as these sows could contaminate ground and provide source of infection for several litters of pigs.



#### D. Investigations of *Trichinella spiralis*.

In 1961 at the Beltsville Parasitological Laboratory, it was reported that, although the standard digestion technique is considered to be a very dependable method for detecting trichinae in meat samples, it is time-consuming and requires relatively expensive and elaborate apparatus. A less complicated and more rapid method was therefore developed in which the psoas muscle of pigs infected with trichinae was blenderized, passed through a 100-mesh screen, and the sediment examined with a dissecting microscope. In this test trichinae were detected in a 20-gram sample of the psoas muscle of an 80-pound pig that had received 80 cysts of *Trichinella spiralis*. The digestion technique was then employed to determine the distribution of trichinae in 200-pound pigs that had received 10 to 100 cysts. Muscles of the neck, tongue, cheek, shoulder, loin, ham, diaphragm and chest wall, and the psoas muscle, were examined. More larvae per gram of tissue were usually found in the cheek, diaphragm, and tongue than in the other tissues, but no consistent pattern of distribution was noted.

In 1962 no work was reported for this project.

#### E. Effect of anthelmintic treatment on rate of gain.

In 1961 and 1962, at Tifton, Georgia, the effect of anthelmintic treatment (sodium fluoride against *Ascaris suum*) on rate of gain when administered to parasitized pigs of different ages and on different levels of nutrition was less than the effect of the age of the pigs and the levels of nutrition. This was attributed mainly to the low rate of parasite infection.

#### F. Pathogenic role of the intestinal roundworm.

In 1961, in research studies under a cooperative agreement with the Nebraska Agricultural Experiment Station, Lincoln, trypsin and chymotrypsin inhibitors were extracted from the body fluid and body wall of adult ascarids. Six-month-old pigs were as readily infected with ascarids as were five-week-old pigs. Liver lesions caused by migrating *Ascaris* healed within 21 days. *Ascaris* larvae migrated readily through the liver and lungs of pigs which had been given two previous exposures of infective *Ascaris* eggs. Thiabendazole, at 0.25 percent of a milk diet, greatly reduced the number of *Ascaris* larvae within the liver and lungs of 4 pigs. *Ascaris* infections have occurred in all of 30 herds of repopulated specific pathogen-free (SPF) pigs.

In 1962 at the Nebraska Station, subcutaneously injected thiabendazole effectively stopped migrating *Ascaris suum*. Hygromycin B, and a cadmium formulation effectively removed adult ascarids from swine. A N-Benyl derivative of Hygromycin was ineffective. A special formulation of an organic phosphate, 2,2 Dichlorovinyl dimethyl phosphate, was highly efficacious against *A. suum* and *Trichuris suis* in swine. Guinea pigs demonstrated a good immunity to *A. suum* when injected with infective eggs and specific protein components of the adult worms.

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